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body weights	light scattering sensor
clinical chemistry	lung weight
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clinical pathology	mortality
differential counts	multivariate analysis
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exposure concentration	obscurant
flow-volume	particle dispersion
food consumption	phagocytosis
fractional factorial design	pressure-volume
frequency	pulmonary function
gas dilution	pulmonary lavage
graphite	spontaneous breathing measurements
hematology	statistical evaluation
histopathology	test article
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duration are unrelated to outcome. A notable exception were the pulmonary lavage parameters. The mean aerosol mass concentrations for the study were close to the target levels for all exposure conditions, and particle sizes were in the inhalable range with MMADs of the graphite aerosols ranging from 1.2 to 1.6  $\mu\text{m}$  with a GSD of 2.4 to 3.1. There were no mortalities and clinical observations provided no evidence of significant treatment-related toxicity. Overall, this four-week inhalation exposure had no significant dose-, duration-, or frequency-related effect on body weight gain, food consumption and clinical pathology parameters. Significant concentration-related changes were obtained from the four pulmonary function tests (flow-volume, pressure-volume, gas dilution and tidal breathing tests) in rats exposed to the graphite aerosols compared to the filtered air controls but no important pulmonary function effects related to sex, or the duration and frequency of exposure were found. Pulmonary lavage of rats inhaling graphite aerosol showed increased numbers of cells, increased amounts of protein and altered types of pulmonary cells. Statistical analysis revealed that these data were affected in various ways by exposure duration, frequency and concentration, respectively.

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## FOREWORD

This report entitled "Inhalation Toxicity of Single Materials and Mixtures: Phase II - Four-Week Inhalation Toxicity Study of a Solid Particulate Aerosol in F344/N Rats", describes studies conducted by the Life Sciences Research Department of IIT Research Institute for the U. S. Army Medical Research and Development Command under Contract No. DAMD17-89-C-9043 (IITRI Project L06234). The report covers activities for the period of September 1990 through March 1991. Dr. Jack Dacre was the Contracting Officer's Technical Representative. Catherine Aranyi, Principal Investigator, was in charge of the overall conduct and coordination of the program. Jeannie Bradof, Study Director, was responsible for incorporating the complex experimental design features required for these studies into an operational protocol, and for its supervision and promulgation. Drs. Robert Gibbons, and Don Hedeker, Consultant Biostatisticians, provided the statistical design for the program and conducted the overall statistical evaluation of the studies. Dr. Narayanan Rajendran, Co-Investigator, was in charge of the test atmosphere generation and monitoring system and the conduct of the aerosol exposures. In addition, the following individuals were in charge of various study areas and contributed to this report: Dr. David McCormick - toxicology; Dr. Barry Levine, Consultant and Mary Ann Cahill - clinical pathology; Dr. M. Tomlinson - histopathology; Dr. Robert Sherwood - pulmonary lavage studies; Dr. Jeffrey Tepper and Michael Stevens, Consultants - pulmonary physiology.

This report is organized in two parts in order to aid the reader in locating and reviewing principal subject areas. Part One entitled "Experimental Program" summarizes the design, conduct and results of the experimental phases of the program. Appendices to Part One include experimental data tables; the pathology report, and the study protocol in toto. The statistical report is presented in Part Two and consists of a summary discussion entitled "Statistical Overview of the Results", with the various subject areas (body weights; food consumption; lung/body weight ratios; clinical chemistry; hematology; differential counts; pulmonary lavage parameters; pulmonary function) included in Appendices following the main section.

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CA In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

NA For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institute of Health.

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Catherine Aranyi, PI                      Date

## GLP COMPLIANCE STATEMENT

This study was conducted in accordance with U. S. Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards as set forth in the Code of Federal Regulations (Part 792 of Title 40; TSCA), except that all chemical analyses pertaining to the characterization, stability and homogeneity of the bulk test article and attendant documentation were the responsibility of the Sponsor. There were no significant deviations from the aforementioned GLP standards that would have affected the integrity of the study or the interpretation of the test results. The raw data have been reviewed and the information contained in this report is an accurate representation of the data within the context of the study design and evaluation criteria.

Study Director:

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Jeannie Bradof  
Research Toxicologist  
Life Sciences Research

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Date

## EXECUTIVE SUMMARY

This study supported by the U.S. Army Biomedical Research and Development Command is conducted to investigate the potential inhalation hazard of materials employed as obscurants to which military personnel may be exposed during field operations. The overall objective is to evaluate the toxicity of aerosols and mixtures of aerosols by exposing laboratory rats in whole-body inhalation chambers under simulated field conditions. The program requires evaluation of the potential inhalation hazards of aerosols of a solid particulate material, graphite, and mixtures of this solid particulate aerosol with a petroleum-based liquid aerosol. The subject of the current Phase II report is examination of the effects of inhalation of aerosols of the solid particulate test material. This report is presented in two parts. Part One entitled "Experimental Program" summarizes the design, conduct and results of the experimental phases of the program. Appendices A, B and C to Part One include experimental data tables; the pathology report and the study protocol in toto. The statistical report is presented in Part Two entitled "Statistical Overview of the Results", followed by an Appendix with the specific discussions and associated tables in various subject areas included in Sections A through H.

According to the objectives of Phase II, a four-week inhalation toxicity study to aerosols of graphite particles was conducted to evaluate the effects of aerosol exposure concentration, daily duration, and weekly frequency on selected biologic endpoint parameters in male and female F344/N rats; to establish if sex affected the results; and to evaluate the impact of a recovery period. Biologic endpoints included pulmonary lavage parameters, pulmonary function, lung weights, histopathology and clinical pathology. These endpoints were evaluated within 24 hrs after the last exposure and after a two-week recovery period. In addition, all animals on test were monitored throughout the study for in-life clinical signs, body weights, and selected groups for food consumption. A fractional factorial design, which allows for the most efficient use of the experimental resources, was used for the statistical evaluation of the studies.

The facilities dedicated to these studies include two inhalation exposure laboratories provided with conditioned air supply and chamber air exhaust systems; inhalation exposure chambers with air flow and pressure controls; and aerosol generators provided by the Government. There are seven  $1\text{m}^3$ -size inhalation chambers: four were used for exposure to aerosols of the graphite test material, and one for the positive control aerosol. Two chambers in a separate room were used to expose the control animals to filtered air. A two-stage filtration system, consisting of a bag prefilter and a HEPA filter, were used to exhaust the chamber atmosphere.

The exposures were conducted at two target concentration levels (100 and  $200\text{ mg/m}^3$ ) of the graphite aerosol for two exposure durations (1 and 4 hrs/day) and two exposure frequencies (2 and 4 days/wk). In

addition exposures to cristobalite aerosol (positive control material) were conducted at one concentration level ( $200 \text{ mg/m}^3$ ) for 4 days/wk and 4 hrs/day. The aerosol test atmospheres of both the graphite and cristobalite powders were generated with pneumatic aerosol generation systems provided by the Government. Each of the exposure chambers was interfaced with a separate aerosol generation system. The generation system consists of a jet mill and a screw feeder. The screw feeder meters the test material into the jet mill which aerosolizes the powder using compressed air. The aerosol concentration in the exposure chambers was monitored using gravimetric filters collected once per exposure hour and the concentration variations were monitored in real time with Portable Continuous Aerosol Monitors. (Details of the test atmosphere generation and monitoring methods were described in our Phase I Report submitted for this contract.)

The overall mean aerosol concentrations for the study were generally close to the target levels, ranging from 94.4 to 99% for all exposure conditions. The overall Relative Standard Deviation (%RSD) of the concentrations ranged from 2.7 to 9.2%. The daily variation of aerosol concentrations (daily %RSD) in all the chambers was below 10% except on few occasions but never higher than 16.1%. Mass Median Aerodynamic Diameters of the graphite aerosols ranged from 1.2 to 1.6  $\mu\text{m}$  with a Geometric Standard Deviation of 2.4 to 3.1.

The experimental design for this study was prepared in cooperation with Dr. R. Gibbons, biostatistical consultant to the program, according to our contractual agreement with the Government. Because of the complex experimental design requirements mandated by the RFP and the physical limitations (equipment and personnel) of the experimental logistics that required the simultaneous testing of such a large number of conditions under a rigorous schedule, a fractional factorial statistical design was selected. The fractional factorial design allowed the evaluation of all possible main effects and interactions of the three primary factors and all two-way interactions involving sex and recovery. This design, in conjunction with statistical power computations based on historical data, allowed the use of relatively low group sizes in combination with an "experimental endpoint day" strategy limited to 4 days after the last exposure and 4 days after the recovery period.

Multivariate analysis of variance models were used to analyze each set of variables (i.e., body weight gain, food consumption, relative lung weight, clinical chemistry, hematology, hematology/differential counts, pulmonary lavage, and pulmonary function.) Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: sex, period (post-exposure or post-recovery), concentration (filtered air control, 100 and  $200 \text{ mg/m}^3$  graphite aerosol, or positive control aerosol at  $200 \text{ mg/m}^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner which would allow tests of all two-way interactions to be made.

Multivariate analysis of variance models were used to analyze the body weight gains and food consumption. (Dr. Gibbons' detailed report on the statistical evaluation methods and results are presented in Part Two.)

The results of the statistical computations revealed a remarkably striking absence of significant main effects of any of these factors, suggesting that differences in levels of concentration, frequency, and duration are unrelated to outcome. A notable exception, however, was the pulmonary lavage parameters. Here, several measurements were affected by all three factors, either significantly increased or decreased.

There were no mortalities, and clinical observations provided no evidence of significant treatment-related toxicity in any experimental group during either the exposure or recovery periods. Overall, the four-week inhalation exposure to the graphite aerosols had no significant dose-, duration-, or frequency-related effect on body weight gain. Although statistically significant differences were detected in a number of individual comparisons as described in the Part Two Statistical Report, the overall results of the study provided little evidence to support the hypothesis that increased exposure to the test article via increased exposure concentration, duration, and/or frequency was associated with significant effects on body weight. Because no consistent, dose-related alterations of body weight gains were observed in groups exposed to the graphite aerosols, it is concluded that the test article had no biologically significant impact on this parameter.

All groups exposed to the graphite or the positive control aerosols demonstrated statistically significant decreases in food consumption when compared to filtered air controls. However, in groups exposed to the graphite aerosol, these decreases were not dose-related, and no consistent effect of concentration, duration, or frequency of exposure were observed.

Neither concentration, duration, nor frequency of exposure of the graphite aerosols affected clinical pathology parameters. Sporadic increases and decreases were seen which were not considered biologically significant. For the positive control group, total WBC counts were slightly, but significantly, elevated at the post-exposure timepoint for both sexes. These elevations were primarily due to increased numbers of neutrophils. Recovery from neutrocytosis was not apparent at the post-recovery timepoint. No other clinical pathology measurements were altered for the positive control group.

Histopathologic evaluation revealed no microscopic lesions related to the test article at the post-exposure or post-recovery time points in animals that had been exposed to the most severe conditions of concentration, duration or frequency. Animals exposed to the positive control, crystalline silica, developed granulomatous inflammation in lungs and in pulmonary and mediastinal lymph nodes. In animals exposed to the test article, pigment was seen within alveolar macrophages or within macrophages in the interstitium and lymphoid

tissue of the lung. The presence of pigment in these locations is interpreted as evidence of exposure of the test article but not as a test article-related lesion.

Although, the overall effects of inhalation of the graphite particles on relative lung weights are inconsistent, some observations in the high concentration/frequency/duration groups deserve to be noted, as well as the fact that more of these changes appeared post-recovery. These data should be viewed in parallel with the outcome of the lavage studies.

Results of the pulmonary lavage studies indicate that inhalation exposure to graphite aerosols did not alter cellular viability or adversely affect fc-mediated phagocytic activity of alveolar macrophages. This suggests the lack of toxicity to cellular functions from the inhaled graphite aerosols. Pulmonary lavage of graphite-exposed rats indicated the presence of increased numbers of cells, increased amounts of protein and altered types of pulmonary cells and statistical analysis revealed that these data were affected in various ways by exposure duration, frequency and concentration. These changes are consistent with the effects of a pulmonary irritant. All parameters, except for total cells and total viable cells, were within normal values after a two-week recovery.

Tidal breathing, gas dilution, pressure-volume and flow-volume pulmonary function tests were performed both immediately post-exposure and after a two-week post-recovery period. Several parameters were obtained from each of these four tests. Every test showed one or more parameters that were significantly affected by the test aerosol or showed strong concentration-related trends. Overall, the tests indicated a mild restrictive lesion was developing immediately post-exposure, that partially resolved during the post-recovery period. Tidal breathing tests indicated that tachypnea, a pattern of breathing consistent with restrictive lung disease occurred. The gas dilution test revealed that lung volumes and diffusing capacity were reduced, indicating a stiffer and thickened interstitium, respectively. Compliance, obtained from the pressure-volume curve and during tidal breathing was reduced post-exposure, revealing that more pressure was required to expand the lung than normal. Finally, lung volumes were reduced in exposed rats during the latter portion of the flow-volume curve without a reduction in air flow. This pattern of response would also suggest a mildly restricted lung. The data obtained from the lavage fluid indicating neutrophil influx and accumulation of protein would suggest that a chronic inflammatory response may be responsible for the pulmonary function observations. However, since the inflammatory response was greater for the positive control exposure and the pulmonary function response was somewhat less, inflammation cannot be totally explanatory. Overall, the multivariate fractional factorial analysis did not detect any important pulmonary function effects related to the sex of the animals, or the duration and frequency of exposure. Furthermore, the analysis revealed that many of the altered pulmonary measurements recovered to within normal limits during the post-recovery period. Thus, this study failed to detect any significant effects of the factors of sex, frequency and duration on pulmonary function parameters.

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INHALATION TOXICITY OF SINGLE MATERIALS AND MIXTURES:  
PHASE II - FOUR-WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS

PART ONE

EXPERIMENTAL PROGRAM

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MARCH 29, 1991

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## I. INTRODUCTION

The U.S. Army Research and Development Command is concerned for the safety of military personnel during field operations when they may be exposed to high concentrations of airborne materials for short daily durations repeated over irregular periods for a number of weeks. Since the airborne materials may be present as single materials and/or as mixtures of materials, the objective of this program is to evaluate the toxicity of test atmospheres consisting of such aerosols and aerosol mixtures by exposing laboratory rats in inhalation chambers under simulated field conditions.

This four-week inhalation toxicity study to aerosols of a solid particulate test material (graphite) was conducted to evaluate the effects of aerosol exposure concentration, daily duration, and weekly frequency on selected biologic endpoint parameters in male and female F344/N rats; to establish if sex affected the results; and to evaluate the impact of a recovery period. Biologic endpoints included pulmonary lavage parameters, pulmonary function, lung weights, histopathology and clinical pathology. These endpoints were evaluated within 24 hrs after the last exposure and after a 2-week recovery period. In addition, all animals on test were monitored throughout the study for in-life clinical signs, body weights, and selected groups for food consumption. A fractional factorial design, which allows for the most efficient use of the experimental resources and multivariate analysis of variance models, were used for the statistical evaluation of this study. Results will be used to select a single daily duration and one weekly exposure frequency for the next study which will be conducted with complex aerosol mixtures of this solid and a petroleum-based liquid test material.

## II. MATERIALS AND METHODS

### 1. INHALATION EXPOSURES

#### 1.1 Test Materials

The exposure study was conducted with graphite powder using crystalline silica (cristobalite) as positive control. The graphite (Powder A1) test article (received August 31, 1989) was provided by the Government and the cristobalite (received on September 21, 1990) was procured from the CED Corporation, Ohio. All test articles were stored under ambient condition until used in the aerosol generators.

#### 1.2 Inhalation Exposure Facilities

The facilities dedicated to these studies include two inhalation exposure laboratories (Laboratories I and II) provided with conditioned air supply and chamber air exhaust system; inhalation exposure chambers with air flow and pressure controls; and aerosol generators provided by the Government. There are seven Rochester-type, one-cubic-meter inhalation chambers; five of which are located in Laboratory I and are used for exposure to aerosols of the test materials. Two chambers are in a separate room (Laboratory II) for exposure of the control animals to filtered air and to prevent contamination and contact of these control animals with the test materials. Laboratory I, used for the aerosol exposures, is maintained at a negative pressure relative to the access corridor and the adjoining Laboratory II which contains the chambers for exposure of the control rats to filtered air.

Supply Air: Both laboratories share the same supply air but are connected to separate exhaust systems. Single-pass conditioned air is introduced into the rooms at the rate of 18 to 20 changes per hour. The conditioned room air is introduced into the chambers through individual inlet filter assemblies consisting of a fiberglass coarse filter and a HEPA filter. Before entering the system, supply air passes through particulate prefilters, charcoal filters, and is preconditioned with a water-cooled air conditioning unit. Temperature and humidity are adjusted to maintain conditions of 23 to 27°C and 40 to 70% relative humidity (RH). An electric duct heater with an automatic control system maintains the required temperature range. Two steam humidifiers, one located at the air conditioning unit outlet and the other in the air inlet duct to the laboratories, supply the humidity which is controlled with a high-limit, 85%, pneumatic modulating controller. An automatic air handling control panel for regulating cooling, heating, and humidity is located in Laboratory I. The air supply is capable of providing a per chamber flow rate of 0.5 equivalent volumes per minute (500 l/min) and meets the minimum flow rate requirement of 0.4 equivalent volumes/min necessary for the study.

Exhaust System: The exhaust from each of the aerosol exposure chambers is filtered with a two-stage filtration system and exhausted. The combined exhaust from the five chambers is moved by a pressure blower (2 hp, 3500 rpm electric motor) capable of providing >500 l/min. air flow to each of the experimental chambers against 75 cm of water pressure and is exhausted above the roof outside the building. The individual chamber air flow is controlled with a gate-valve located in the filtered exhaust and monitored with a calibrated orifice meter for each chamber.

The blower is remotely located to minimize noise in the exposure laboratory. The blower is connected to an emergency power supply. An alarm system installed in the exhaust air system provides warning in case of blower failure.

The exhaust system for the filtered air control chambers is independent of the system for the experimental test chambers to avoid potential contamination with the test aerosols. Both exhaust systems are operated continuously except during chamber cleaning or maintenance.

Exhaust Filter System: Air exhausted from each exposure chamber is filtered through a bag-type prefilter followed by a high efficiency particulate (HEPA) filter. The prefilter (Cambridge Model 3295 fiberglass filter) consists of five (5) bags or envelopes mounted in a parallel configuration. The filter has a rating of 93 - 97% efficiency against atmospheric dust, and a holding capacity of 1 gram/cm<sup>2</sup>. The filter is mounted in an epoxy-coated plywood housing. The backup HEPA filter is also from Cambridge Filter Co. (Model 242412, Silver Seal). A Magnehelic pressure gauge is used to monitor pressure drop across these filters and determine the filter loading and the need for filter change.

### 1.3 Aerosol Generation System for Solid Particulates

The aerosols of the graphite powder and cristobalite (positive control material) were generated using aerosol generators provided by the Government (J. H. Moneyhun, T. M. Gayle, and R. A. Jenkins "A system for Generating Mixed Aerosols from a Petroleum-Based Liquid and a Solid", ORNL Draft Final Report). The system consists of a jet mill dispersing unit (Jet-O-Mizer Model 00 Fluid Energy Processing and Equipment Company, Hatfield, PA) fed by a screw feeder (Series 100 Accurate, Whitewater, WI). The rate of dispersion is controlled by the revolution rate of the feeder screw. Solids delivered by the screw feeder into the jet mill funnel are drawn into the mill by aspiration and are accelerated to high velocities by two air jets supplied with compressed air at 100 psi. The particles are swept into turbulent motion and pulverize each other. Consequently, a relatively highly dispersed aerosol is produced at the outlet of the jet mill.

The screw feeder metering accuracy is improved by the stirrer that moves up and down over the feed screw. The stirrer is vibrated by an air driven vibrator. A second vibrator attached to the delivery tube

helps prevent packing in the delivery tube. The delivery rates from the screw feeder were constant and varied only by a few percent (See calibration tables for screw feeder in Appendix A of Phase I report).

Typically, the aerosols generated with jet mills are highly charged electrically and it is customary to neutralize these particles before they enter the exposure chamber. However, the generators used in this study have no provision to neutralize the electric charge. Our discussions with ORNL personnel (developers of the generators) revealed that the graphite test material dispersed under field conditions carries an electric charge and therefore, the laboratory aerosol generators which were designed to simulate the field aerosol had no provisions for aerosol charge neutralization. It is noted that the electric charge on the aerosol modifies the mobility of the particles and alters the deposition onto surfaces.

#### 1.4 Monitoring the Aerosol Test Atmosphere

Monitoring the concentration of an electrically charged aerosol in real-time presents a major technical problem; the electrical charge on the aerosol particles modifies their mobility and they tend to deposit on sensing/optical surfaces and produce measurement artifacts leading to a steady drift in the sensor response. Therefore, the real-time sensors are useful only in establishing relative changes and cannot be used to determine the absolute mass concentration. In view of these difficulties associated in monitoring the electrically charged aerosol using real-time monitoring sensors, the gravimetric method was chosen to be the primary method to monitor the aerosol concentrations. The real-time aerosol sensors were used as on-line guides to keep the aerosol concentrations at target levels. The aerosol particle size was monitored with Quartz Crystal Microbalance (QCM) and Mercer cascade impactors.

Exposure Chamber Homogeneity: The spatial uniformity and temporal stability of the aerosol mass concentration and particle size in the exposure chambers were determined during Phase I of this study and is the entire subject of the Phase I report (submitted in February 1990). A brief description of the method is provided below (For a complete description of the methods and discussion of data, see Phase I report). Spatial and temporal homogeneity of the chamber test atmospheres was established through a procedure of simultaneous sampling from ten (10) locations within the chamber. Aerosol mass concentration and particle size were measured at each target level in all the chambers as a function of location and time. Statistical analysis of the data revealed that the variation of the aerosol concentration was within the required 20% limit in every chamber at all the target concentration levels.



Aerosol Mass Concentration: Aerosol mass concentration was monitored gravimetrically, approximately once for each hour of the exposure period, and a real-time aerosol sensor (PCAM) was employed to keep the concentrations on target.

The real-time sensor used to maintain the chamber aerosol concentration of solid particulates at target level is the Portable Continuous Aerosol Monitor (PCAM) real-time aerosol sensor (PPM, Inc., Nashville, TN). The PCAM Model TX is a microprocessor-based electrooptical system which measures aerosol concentrations by the principal of near forward scattering of light emitting diode radiation. The concentration range of the sensors is 0-200 mg/m<sup>3</sup>. The sensors have an internal self-calibration cycle that occurs once every hour. As discussed earlier, the graphite particles, due to the electrical charge on them, could not be kept away from the optics of the sensor and as a result the sensor's calibration could not be maintained. Therefore, the PCAM sensor was used only as an on-line guide for maintaining the particulate aerosol concentrations on target.

Aerosol mass concentration was determined by gravimetric method. Particles of the test aerosol were collected on pre-weighed 45-mm fiberglass filter disks placed in acrylic plastic filter holders. The filters have a 99.99 percent retention efficiency for dioctyl phthalate particles of 0.3  $\mu$ m. Prior to use, the fiberglass filters were maintained for 24 hours in the conditioned atmosphere of the sampling environment to assure moisture equilibration by the filter pads. The aerosol samples were collected at constant flow rates, using diaphragm-type vacuum air pumps. The filters were weighed on an analytical balance. Dry gas meters connected to the backside of the pumps recorded the corresponding total volume of air sampled. All filter samples were weighed within 30 minutes of removal from the sampling ports, transferred to plastic petri dishes, and entered into a permanent record.

Aerosol Particle Size: The aerosol particle size distribution was monitored by a piezoelectric microbalance-based 10-stage cascade impactor. The Quartz Crystal Microbalance (QCM) is a cascade of aerodynamic-inertial impactors (California Measurements, Sierra Madre, CA), in which the suspended particles are classified according to their effective aerodynamic sizes and weighed in situ and in real time on the impaction surface. This is accomplished by using high-frequency, resonating piezoelectric crystals as the impactor plates. A built-in pump samples an aerosol stream at a rate of 0.24 liters/min, separating the aerosol particles into 10 sequential size ranges from 0.07 to 35.4  $\mu$ m in aerodynamic diameter.

For the cristobalite aerosol, the QCM was not able to measure the particle size because the particles did not adhere well to the sensing crystals. Hence, another cascade impactor (Mercer Cascade Impactor, In-Tox Products, Albuquerque, NM) was used to measure the size. The Mercer impactor has seven stages and operates at a flow rate of 2 l/min and can measure particles in the range of 0.5 to 8.0  $\mu$ m diameter. The material collected in each stage was determined gravimetrically.

## 2. ANIMALS AND MAINTENANCE

### 2.1 Receipt, Quarantine, and Disease Screening Procedures

Two hundred ninety-two male and 292 female F344/N rats were obtained from Taconic Farms Inc., Germantown, NY. on September 18, 1990. The animals were 4-5 weeks of age at arrival and 6-7 weeks of age at the start of the study. The weight ranges for male and female rats at arrival were 62 to 101 g and 57 to 97 g, respectively.

The animals were observed daily during quarantine. Following randomization, two animals per sex were selected from the excess stock for quarantine sacrifice. These animals were bled and sera sent for a standard rat virus profile (Microbiological Associates, Rockville, MD) followed by a gross necropsy. No lesions were discovered during the quarantine necropsy and the test results on all sera were negative.

### 2.2 Randomization

Rats were assigned to groups prior to exposure initiation using a stratified weight method whereby animals were ranked in order, by weight, and assigned to study groups in random order. Weight ranges at randomization were 84 to 140 g for male rats (weighed on September 26 and randomized on September 28, 1990) and 80 to 114 g for female rats (weighed on September 26 and randomized on September 27, 1990). Each rat used in the study was identified by tail tattoo representing a unique number within the population making up the study.

### 2.3 Housing

The animals were housed in stainless steel wire-mesh inhalation cages suspended over excrement pans lined with deotized cage boards (Shepherd Specialty Papers, Inc., Kalamazoo, MI) on mobile racks which were equipped with an automatic watering system. Each mobile rack held 24 cage units and each cage unit contained four individual cubicles for a total capacity of 96 animals per rack. Each cubicle measured 18.4 x 16.5 x 15.9 cm. The animals were double-housed upon arrival to help them become acclimated to their new surroundings and to help them learn to use the automatic watering system. The rats were housed individually at the time of making group assignments and remained individually housed throughout the course of the study. For exposure, the cages were removed from the racks and the rats were moved in their cages into the inhalation chambers. The animals were transferred to clean cages weekly and the cage boards were changed three times per week.

### 2.4 Food and Water

Purina Certified Rodent Chow No. 5002 (Lot No. July 02901B) and filtered City of Chicago drinking water were available ad libitum during nonexposure hours only.

## 2.5 Environmental Conditions

Lighting in the animal rooms was maintained on a 12 hour light/dark cycle. The air supplied to the animal rooms and exposure laboratories was 100% fresh filtered air and provided a minimum of 10 complete air changes per hour. The temperature and relative humidity of the animal rooms were monitored and recorded twice daily on weekdays and once daily on the weekends. The laboratory was regulated such that extreme fluctuations between the animal rooms and the exposure chambers would be avoided. The laboratory parameters were monitored continuously using a drum thermohygrometer. The exposure chambers were monitored every 30 minutes during exposure using a hand-held thermohygrometer. The following is a summary of the environmental conditions:

	Temp °C		% RH	
	Mean	Range	Mean	Range
Exposed Animals:				
Animal Room 1F3-1	24	22-27	32	20-60
Inhalation Chambers 1-5	23	21-26	44	35-52
Control Animals:				
Animal Room 1F3-2	21	18-24	50	32-74
Inhalation Chambers 6-7	23	22-26	48	36-55

## 3. BIOLOGIC ENDPOINTS

### 3.1 Toxicology

Mortality and Clinical Observations: All animals were observed twice daily on weekdays (once daily on the weekends) for moribundity and mortality. Each animal was formally examined twice weekly, once in the morning (before the week's first exposure) and once in the afternoon (after the week's last exposure) for clinical signs of pharmacologic and toxicologic effects of the exposure.

Body Weights: The body weights were measured and recorded individually to the nearest whole gram in the morning prior to exposure at study initiation, twice weekly during the four-week exposure periods, on a weekly basis during the subsequent recovery periods, and immediately before termination. Statistical comparisons were conducted using the sum of the two gains calculated each week on the basis of the biweekly weights during the exposure period, and the weekly measurements during the recovery period.

Body weight gain calculations were based on animal body weight at the beginning of each week to facilitate analysis of weekly food consumption data and its relationship with body weight gain during the same period.

Food Consumption: Average food consumption for each animal in the PATH "recovery" groups (for definitions see Experimental Design Section) was measured for one three-day and one four-day session each week during the four exposure and two recovery weeks of the study. A measured amount of food was offered to the rats on the same days that body weights were scheduled for the exposure portion of the study (or

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on the first day and fourth day of the first and second weeks of the recovery period). On the next scheduled body weighing day (or on the fourth day of the week during the recovery period), the remaining food was weighed and replaced with another measured amount of food. The food consumption was calculated from the measured differences between the provided and recovered food quantities.

Necropsy and Histopathology: Animals remained on exposure regimen until the day prior to (within 24 hours) sacrifice. For all scheduled gross necropsies, specific necropsy procedures were followed. The tissues that were collected and placed in fixative included the upper and lower respiratory tract (nasal turbinates, larynx, trachea, lungs, and pulmonary lymph nodes), heart, liver, spleen, kidneys, urinary bladder, stomach, and adrenals. All tissues and/or organs were examined in situ, then dissected from the carcass, re-examined, including cut surfaces, and fixed in 10% formalin. Tails used for identification were also saved in formalin.

Histopathologic evaluation was conducted on all protocol-specified tissues for those rats exposed to the highest dosage based on concentration and frequency, air control, and positive control animals. As appropriate, if significant treatment-associated tissue changes were found in the high-dose group, then the tissues from the next lower dosage group were processed into slides and microscopically evaluated. The tissues were trimmed, embedded, sectioned and stained with hematoxylin and eosin for histopathologic evaluation.

Lung Weights: Lung weights (to the nearest 1.0 mg) were obtained from all PATH-designated animals. Lung weight/body weight ratios were calculated using terminal body weights.

Clinical Pathology: Blood samples were collected via the retroorbital sinus from CO<sub>2</sub> anesthetized pre-designated PATH rats. Blood collection and same-day analysis of the samples for the hematology and clinical chemistry assays shown below was performed in a predetermined random order.

#### List of Hematology and Clinical Chemistry Tests

##### Hematology

- Erythrocyte Count
- Mean Corpuscular Volume
- Mean Corpuscular Hemoglobin (Derived)
- Mean Corpuscular Hemoglobin Concentration (Derived)
- Hemoglobin
- Hematocrit (Derived)
- Erythrocyte Morphologic Assessment
- Leukocyte Count
- Leukocyte Differential Count
- Platelet Count and Morphologic Assessment

### Clinical Chemistry

Total Protein  
Albumin  
Blood urea nitrogen  
Creatinine  
Alanine aminotransferase  
Alkaline phosphatase  
Creatine kinase  
Total bile acids  
Total cholesterol  
Glucose  
Sorbitol dehydrogenase  
Calcium  
Inorganic phosphate  
Triglycerides

Hematologic determinations were performed with a Baker 9000 hematology analyzer. Quality control assays including abnormal low, normal, and abnormal high (Baker Haem-QC Lot Nos. 0213B-1, -2, -3, 0237B-1, -2, -3, normal, low and high, respectively, for all lots) were run twice each day, prior to initiation of test sample assays and again at the end of the day. Clinical chemistry tests were performed with a Beckman Synchron CX5 analyzer. Normal and abnormal quality control samples were run at the beginning of each daily run and before every eighteenth sample (approximately) throughout the day using Beckman Synchron 1 (Lot No. M906064), Synchron 2 (Lot No. M906065), Synchron 3 (Lot No. M906066), Sigma SDH normal (Lot No. 119F6125), and Nycomed Diagnostics (Norway) Seronorm (Lot No. 177 and 181) and Pathnorm (Lot No. 21 and 26).

The erythrocyte and the platelet morphologic assessment were done during the differential count of the blood smear stained with Wright-Giemsa stain. At least 100 leukocytes were identified.

### **3.2 Pulmonary Lavage Parameters**

Pulmonary Lavage, Total and Differential Cell Counts: Alveolar macrophages (AM) were obtained by tracheobronchial lavage. The animals were sacrificed by intraperitoneal injection of sodium pentobarbital and the lungs of rats were lavaged in situ with nine consecutive 6-ml infusions of warm saline. The AM were collected from the lavage fluids by centrifugation (approximately 250xg for 10 min at 4°C) and resuspended in RPMI 1640 (Whittaker Bioproducts, Walkersville, MD) + 10% low endotoxin fetal calf serum (Hyclone Labs., Logan, UT) (RPMI-10). The supernatant from the first two washes of rats was saved for protein determination.

Total and viable cell counts were made in a hemacytometer. Cell viability was determined by exclusion of 0.05% trypan blue dye. Determination of the cellular distribution (i.e., percent of AM,

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polymorphonuclear leukocytes and lymphocytes) was made through differential counts of cytocentrifuge preparations of cells fixed in methanol and stained with Wright's stain.

Pulmonary Lavage Fluid Proteins: Lavage fluid total protein was determined by the method of Bradford (Anal. Biochem. 72:248-254, 1976) using Coomassie Protein Assay Reagent (Pierce, Rockford, IL). Triplicate 50  $\mu$ l aliquots of lavage fluids were placed into replicate wells on a 96-well assay plate, 250  $\mu$ l of dye reagent added to each well and absorbance read in an ELISA reader at 600 nm (Bio-Tek EL-310, Winooski, VT). Readings were compared to a standard curve generated using bovine serum albumin standard (Pierce) by reverse linear regression.

Alveolar Macrophage Fc Receptor-Mediated Phagocytosis: Phagocytosis of  $^{51}\text{Cr}$ -chicken red blood cells (CRBC) was determined by using a modification of the method of Smialowicz et al. (Environ. Res. 33:413, 1984). Macrophages ( $5 \times 10^5$  AM) were incubated, in triplicate per cell pool, in the presence of the optimal concentration of anti-CRBC antisera with  $^{51}\text{Cr}$ -CRBC at a 10:1 CRBC to macrophage ratio for 1 hr at 37°C. Non-engulfed CRBC were lysed with lysing buffer (8.29 g  $\text{NH}_4\text{Cl}$ ; 1.0 g  $\text{KHCO}_3$ ; 0.0372 g  $\text{Na}_2\text{EDTA}$  per liter, pH 7.4) and removed before the macrophage-associated  $^{251}\text{Cr}$ -CRBC were counted in a gamma counter (Gamma Trac 1191; TM Analytic, Elk Grove Village, IL).

### 3.3 Pulmonary Function

Preparation: Prior to testing, the rats were anesthetized with 40 mg/kg sodium pentobarbital (IP) and a cannula was transorally inserted into the trachea using an illuminated small animal laryngoscope. The rats were placed in a whole-body flow plethysmograph (BUXCO Electronics, Inc.) for lung function measurements. Before sealing the plethysmograph, an infant feeding tube, filled with saline was inserted 7-8 cm down the esophagus.

Data Collection: Analog outputs from precalibrated transducers were digitized by an on-line LS-14 microcomputer (BUXCO Electronics, Inc.) and displayed on a scrolling video monitor. This computer was also used to compute the various measurements of pulmonary function (BUXCO Electronics, Inc.) and send the data to a second computer via the LANtastic high speed network operating system (Artisoft, Phoenix, AZ). The second computer served as the experimenter interface to start maneuvers, monitored the progress of each test and display the results.

Spontaneous Breathing Measurements: Initially, the spontaneous tidal breathing pattern was monitored to obtain measurements of tidal volume ( $V_t$ ), frequency of breathing (FOB), maximal tidal inspiratory and expiratory flows ( $V_{\text{imax}}$ ,  $V_{\text{emax}}$ ) as well as breath timing parameters, inspiratory ( $T_i$ ), expiratory ( $T_e$ ) and relaxation times (RT).

Simultaneously, breathing mechanics were monitored and measurements of esophageal pressure (Pes), as an analog of pleural pressure, lung resistance (Rl) and dynamic compliance (Cdyn) were obtained.

Lung Volume Measurements by the Gas Dilution Method: Vital capacity (VC) was measured between airway pressures of -15 and +30 cm H<sub>2</sub>O pressure. Residual volume (RV) was obtained by gas dilution method using 0.5% neon (Takezawa et al., 1980). Total lung capacity (TLC) was computed as the sum of VC and RV. Multi-breath diffusing capacity of carbon monoxide (DLco) was also obtained during the gas dilution procedure using 0.5% carbon monoxide (Takezawa et al., 1980).

Pressure-Volume Measurements: In order to give all rats a similar volume history, the rats were slowly inflated (3 ml/sec) to TLC and then allowed to relax passively before performing the pressure-volume maneuver. During data collection, again, the rats were inflated to TLC and this time they were slowly deflated to RV (3 ml/sec). Vital capacity (VCpv), the chord slope compliance (Cchord) between 0 and 10 cm H<sub>2</sub>O and the peak compliance (Cpk) were obtained from the pressure volume curve.

Forced Expiration Measurements: Small airway integrity was evaluated during forced expiration. Again the rats were given a constant volume history (see above). During the actual maneuver, the rats were slowly inflated to +30 cm H<sub>2</sub>O and under computer control, a solenoid rapidly opened, exposing the rat's airway to -40 cm H<sub>2</sub>O. From the resultant maximum expiratory flow-volume curve, forced vital capacity (FVC), forced expiratory volume at 50 msec (FEV50), 100 msec (FEV100), 200 msec (FEV200), and 400 msec (FEV400), peak flow (PEXF), volume at peak flow (VPEXF), mean mid expiratory flow (MMEXF), flow at 50% (FEF 50), 25% (FEF 25) and 10% (FEF10) of the remaining FVC were computed.

#### 4. EXPERIMENTAL DESIGN AND STATISTICAL APPROACH

Report by Jeannie Bradof

##### 4.1 Objectives of the Study

This four-week inhalation toxicity study to aerosols of a solid particulate test material (graphite) was conducted to evaluate the effects of aerosol exposure concentration, daily duration, and weekly frequency on selected biologic endpoint parameters in male and female F344/N rats; to establish if sex affected the results; and to evaluate the impact of a recovery period. Biologic endpoints included pulmonary lavage parameters, pulmonary function, lung weights, histopathology and clinical pathology. These endpoints were evaluated within 24 hrs after the last exposure and after a 2-week recovery period. In addition, all animals on test were monitored throughout the study for in-life clinical signs, body weights, and selected groups for food consumption. A fractional factorial design, which allows for the most efficient use of the experimental resources, was used for the statistical evaluation of this study.

## 4.2 Overview of Experimental and Statistical Design

The study consisted of five factors, three primary (concentration, duration and frequency) and two secondary (sex and recovery). The three experimental factors describing the exposure conditions which were used at two levels each, were: concentration (C1 and C2), daily duration (D1 and D2), and weekly frequency (F1 and F2). For rats exposed to aerosols of the test article, there were eight combinations of these exposure conditions. In addition, control rats were exposed to filtered air (C0) or a positive control particle (CP) under the "maximal stress" conditions, i.e., F2D2. Thus, there were a total of 10 treatment groups: eight exposed to the test aerosol at various combinations of C, F, and D and two control groups.

C1F1D1; C2F1D1; C1F2D1; C2F2D1; C0F2D2; CPF2D2;

C1F1D2; C2F1D2; C1F2D2; C2F2D2

The experimental design for this study was prepared in cooperation with Dr. R. Gibbons, biostatistical consultant to the program, according to our contractual agreement with the government. Because of the physical limitations (equipment and personnel) of the experimental logistics that required the simultaneous testing of such a large number of conditions under a rigorous schedule, a fractional factorial statistical design was selected. Dr. Gibbons' detailed report on the statistical evaluation methods and results of these studies are presented in Part Two. Briefly, the fractional factorial design allowed us to evaluate all possible main effects and interactions of our three primary factors and all two-way interactions involving sex and recovery. This design, in conjunction with statistical power computations based on historical data, allowed us to use relatively low group sizes in combination with an "experimental endpoint day" strategy limited to 4 days after the last exposure and 4 days after the recovery period.

More specifically, for the endpoints of pulmonary lavage and pulmonary function, there were three rats of each sex distributed among the various treatment conditions to the test aerosol (one set of conditions per sex). In addition, two filtered air control rats per sex and one positive control aerosol-exposed rat per sex were used per endpoint each day. For the histopathology/clinical pathology group we used three times as many animals, i.e., we had nine rats per sex for the aerosol treatment conditions, six per sex for air controls, and three per sex for positive controls.

Thus for each of the eight "C F D" conditions for test article aerosol exposures, there were 15 designated rats/sex (3 lavage + 3 pulmonary function + 9 pathology) for the terminal as well as the recovery timepoints (one sex/condition/timepoint); for C0D2F2, 40 rats/sex/timepoint, and for CPD2F2, 20 rats/sex/timepoint.



The total number of animals used for the study was computed as follows:

- o 240 test aerosol-exposed rats were distributed into four chambers (Chamber Nos. 1, 2, 3, and 5)
- o 80 positive control rats were exposed in one chamber (Chamber No. 4)
- o 160 filtered air control rats were distributed into two chambers (Chamber Nos. 6 and 7)

#### 4.3 Specific Plan

Groups consisting of 15 male and 15 female F344/N rats were exposed to 100 (C1) or 200 (C2) mg/m<sup>3</sup> aerosols of the test article for 1 hr (D1) or 4 hr (D2)/day, two (F1) or four (F2) days/week for four weeks. A control group of 80 male and 80 female F344/N rats were exposed to filtered air (C0) and a positive control group of 40 male and 40 female F344/N rats were exposed to crystalline silica aerosols at 200 mg/m<sup>3</sup> (CP) for 4 hr/day (D2), 4 days/week (F2) for 4 weeks (Table II-1). Biological endpoints were determined within 24 hr after the last exposure (EXP) and after a 2-week recovery period (REC). The four week exposure period was staggered over five weeks and exposure start dates were staggered over a two-week period to accommodate the four assay days needed at the EXP and REC timepoints. Exposures in the fourth week of exposures were shifted for some groups to accommodate the assay dates. Biological endpoint assays for two Exposure Classes (from classes II through IX) and controls (Exposure Classes I and X) were conducted on each endpoint day.

#### 4.4 Implementation of Design

The inclusion of both two- and four-exposure/week groups in the experimental design, both of which had to be simultaneously evaluated at the same interval (within 24 hr or after a two-week recovery period) following their last exposure on the four assay days at the EXP and REC timepoints, made complex staggering of the exposure starts necessary. Our approach to the problem was to separate the animals into eight different Start Groups or Series. Start Groups 1 to 4 had their first exposure on Monday, Tuesday, Wednesday, and Thursday of the first week when exposures were conducted. Start Groups 5 to 8 similarly had their first exposure on Monday, Tuesday, Wednesday, and Thursday of the second week when exposures were conducted. Thus, rats designated for experimental design groups I, IV, V, VIII, IX, or X (all with exposure frequency = four times/week) were assigned to Start Groups 1 or 5 (exposures Monday through Thursday) or 2 or 6 (exposures Tuesday through Friday) depending on which of the four EXP or REC assay dates they were designated. Similarly, rats designated for experimental design groups II, III, VI, or VII (all with exposure

TABLE II-1.  
EXPERIMENTAL DESIGN<sup>a</sup>

Expo. Class Code	Test Compd.	Expo. Conc. mg/m <sup>3</sup>	Expo. hr/day	Weekly Expo. Freq.	Animals of	In-Life Testing				Post-Exposure Assays <sup>b</sup>						Post-Recovery Assays <sup>b</sup>					
						Daily Clinical		Body		Food Consumpt.	LAV <sup>i</sup>	PF <sup>j</sup>		PATH <sup>k</sup>	LAV <sup>i</sup>	PF <sup>j</sup>		PATH <sup>k</sup>			
						Obs <sup>e</sup>	Signs <sup>f</sup>	M	F			M	F			M	F		M	F	M
I	FAC	0	4	4	80	80	80	80	80	24	24	8	8	8	8	24	24	24	24		
II	A1	100	1	2	15	15	15	15	15	9	9	3	0	3	0	9	0	3	0		
III	A1	200	1	2	15	15	15	15	15	9	9	0	3	0	3	0	9	0	9		
IV	A1	100	1	4	15	15	15	15	15	9	9	0	3	0	3	0	9	3	0		
V	A1	200	1	4	15	15	15	15	15	9	9	3	0	3	0	9	0	3	0		
VI	A1	100	4	2	15	15	15	15	15	9	9	0	3	0	3	0	9	0	9		
VII	A1	200	4	2	15	15	15	15	15	9	9	3	0	3	0	9	0	3	0		
VIII	A1	100	4	4	15	15	15	15	15	9	9	3	0	3	0	9	0	3	0		
IX	A1	200	4	4	15	15	15	15	15	9	9	0	3	0	3	0	9	3	0		
X	PC	200	4	4	40	40	40	40	40	12	12	4	4	4	4	12	12	4	4		

a Numbers represent sample sizes.

b Four assay days occur at each timepoint (EXP = within 24 hr of the last exposure; REC = 14 days after the last exposure) to accommodate all of the treatment groups. Two experimental groups plus positive and negative controls are scheduled for each assay day.

c Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles.

d FAC = filtered-air control; A1 = graphite; PC = positive control, crystobalite.

e Twice/day for mortality and morbidity.

f Twice weekly.

g Twice weekly for the four-week exposure period, weekly during the subsequent two-week recovery period (where applicable) and immediately before termination.

h Weekly for selected animals.

i LAV = Pulmonary lavage assays (see text).

j PF = Pulmonary function assays (see text).

k PATH = Histopathology/clinical pathology assays (see text).

<sup>a</sup> Numbers represent sample sizes.

<sup>b</sup> Four assay days occur at each timepoint (EXP = within 24 hr of the last exposure; REC = 14 days after the last exposure) to accommodate all of the treatment groups. Two experimental groups plus positive and negative controls are scheduled for each assay day.

<sup>c</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles.

<sup>d</sup> FAC = filtered-air control; A1 = Graphite; PC = positive control, crystoballite.

<sup>e</sup> Twice/day for mortality and morbidity.

<sup>f</sup> Twice weekly.

<sup>g</sup> Twice weekly for the four-week exposure period, weekly during the subsequent two-week recovery period (where applicable) and immediately before termination.

<sup>h</sup> Weekly for selected animals.

<sup>i</sup> LAV = Pulmonary lavage assays (see text).

<sup>j</sup> PF = Pulmonary function assays (see text).

<sup>k</sup> PATH = Histopathology/clinical pathology assays (see text).

frequency = two times/week) were assigned to Start Groups 3 or 7 (exposures Wednesday through Thursday) or 4 or 8 (exposures Thursday through Friday). The schedule for body weights, clinical observations, and food consumption measurements was different for each of the eight Start Groups such that the same functions occurred for each group on the same relative study days, with Study Day 1 being the first day of exposures for each group.

### III. RESULTS AND DISCUSSION

#### 1. AEROSOL TEST ATMOSPHERE MONITORING Report by N. Rajendran

##### 1.1 Aerosol Mass Concentration

The graphite (Test Article) and cristobalite (Positive Control) aerosol monitoring results for the study, shown in Table III-1, are presented as overall means of the daily mean aerosol mass concentrations with associated standard deviations (SD) and % relative standard deviations (RSD). The daily mean aerosol mass concentrations were calculated from the filter-collected samples collected once per hour during the various exposure durations. For Chamber Nos. 1 and 2, the aerosol mass concentrations are based on single measurements for each exposure because of the one hour exposure durations. The aerosol concentration measurements for each exposure day (individual measurements for Chambers 1 and 2 which have 1-hr exposure duration and daily means for Chambers 2,3 and 4 which have 4-hr duration) along with daily statistical computations are provided in Tables A-1 to A-5 of Appendix A. These data show that the concentrations were within  $\pm 20\%$  from the target level on all exposure days except one, when the mean in Chamber No. 3 was 22% higher than the target. (The %RSD of the concentrations in any one day was less than 16.2%). To test whether or not the mean aerosol concentration at each target level was the same for each of the test conditions, the t-test was applied to concentration data. The test results (not shown) indicated that there was no significant difference ( $p \leq 0.05$ ) in the mean aerosol concentrations for experimental groups with various weekly exposure frequencies and exposure durations.

The overall mean aerosol concentrations calculated from the daily means shown in Table III-1 were generally close to target levels under all exposure conditions and ranged from 94.4% to 99.0% of the target concentration levels. The %RSDs for the study ranged from 2.7% to 9.2%, well within the required limits of 20%.

The daily variation of aerosol concentrations (daily %RSD) in chambers with 4-hr exposure duration were within the 20% limit on all exposure days. In general, the majority of the %RSDs were well below 10% except on six exposure days when the %RSD ranged from 10 to 16% (For details, see Tables A1 to A5 of Appendix A).

##### 1.2 Aerosol Particle Size

The aerodynamic particle size of the graphite and cristobalite aerosols was measured during the exposure study using a cascade impactor. The particle size distribution parameters of the graphite and cristobalite aerosol in the exposure chambers are exhibited in Table III-2. The Mass Median Aerodynamic Diameters (MMAD) of the graphite aerosols were in the range of 1.2 to 1.6  $\mu\text{m}$  and the geometric standard deviations (GSD) were in the range of 2.4 to 3.1. For the cristobalite aerosol, the MMAD was 2.2  $\mu\text{m}$  with a GSD of 2.3.

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TABLE III-1  
SUMMARY OF AEROSOL MASS CONCENTRATIONS  
4 WEEK EXPOSURE STUDY

Chamber No.	Exposure Duration (hrs)	Target Conc. <sub>3</sub> mg/m	Frequency of Exp. (days/wk)	Aerosol Mass Concentrations <sup>a</sup>			
				Mean <sub>3</sub> mg/m	tsd	N <sup>b</sup>	% of Target (Mean/Target)
1	1	100	4	94.4	8.3	22	94.4
			2	96.5	8.9	11	96.5
5	4	100	4	98.2	7.9	18	98.2
			2	97.3	6.5	13	97.3
2	1	200	4	198.0	15.4	22	99.0
			2	196.2	10.4	10	98.1
3	4	200	4	197.2	11.5	24	98.6
			2	194.7	5.2	11	97.4
4 <sup>c</sup>	4	200	4	195.5	17.3	28	97.8

<sup>a</sup> Determined gravimetrically from filter-collected samples.

<sup>b</sup> Number of exposure days in the study; 2-days/wk data are subset of 4-days/wk data.

<sup>c</sup> Positive control, cristobalite aerosol.

TABLE III-2  
SUMMARY OF AEROSOL PARTICLE SIZE DISTRIBUTION  
4-WEEK EXPOSURE STUDY

Chamber No.	Test Material	Target Conc. $\mu\text{g}/\text{m}^3$	Size Distribution Parameter <sup>a</sup>	
			MMAD <sup>b</sup> , $\mu\text{m}$	GSD
1	Graphite	100	1.2	2.9
2	Graphite	200	1.6	3.1
3	Graphite	200	1.4	2.6
5	Graphite	100	1.3	2.4
4	Cristobalite	200	2.2	2.3

<sup>a</sup> Graphite aerosol size measured with Quartz Crystal Microbalance (QCM);  
cristobalite aerosol size measured with Mercer Cascade Impactor. Numbers  
shown are Average of two measurements.

<sup>b</sup> Mass Median Aerodynamic Diameter and Geometric Standard Deviation.

## 2. TOXICOLOGY

Report by D. McCormick

### 2.1 Survival/Mortality and Clinical Observations

There were no mortalities in any of the exposure groups during this study. Clinical observations were made twice weekly throughout the exposure and recovery periods. Because the results of these observations (Table A-6, Appendix A) demonstrated no significant toxicity, no statistical analysis of the observations was performed.

The vast majority of animals exhibited no clinical evidence of toxicity at any point in the study. No signs were observed in most animals, regardless of exposure protocol; where specific deviations from normal were observed, most were limited to the presence of the test article on the fur or around the nose, or discolored paws. All of these observations are a result of the coloring of the graphite test article, and are unrelated to toxicity.

Red nasal discharge or red fur around the nose was seen in a small number of rats from several groups at various times during the exposure and/or recovery periods. Based on the small number of animals involved, lack of dose-relatedness of the observation, and the relatively non-specific nature of the clinical sign, this observation is not considered to be of toxicologic significance.

Possible more important clinical signs (diarrhea, hunched posture, redness around eyes) were limited to only a single animal for each observation. As such, these observations are not considered to be of toxicologic significance.

In summary, clinical observations provided no evidence of significant treatment-related toxicity in any experimental group during either the exposure or recovery periods.

### 2.2 Body Weight

The influences of exposure to aerosols of the test article and the positive control material on body weight were evaluated using multivariate analysis of variance models comparing weekly body weight gains in treated groups versus those in filtered air controls. Statistical comparisons involving the positive control group were conducted independently from those involving groups exposed to the test article. All rats were weighed twice weekly, and statistical comparisons were conducted using the sum of the gains calculated on the basis of these two measurements.

The staggered start that had to be used in this study because of the complex experimental design required that body weight comparisons be made on the basis of body weight gain, rather than body weight itself. Because the length of the quarantine period for individual animals was varied to accommodate the staggered start, the age range of the experimental population was greater at the initiation of exposure than

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at the time of receipt. Because body weight correlates with age in young adult rats, age differences increase the variability of body weights within the population. This variability decreases the sensitivity of body weight as an indicator of gross toxicity, and as such, decreases the value of comparisons based on this parameter.

By contrast, small age differences have no effect on the rate of body weight gain of animals of the age range used in the study. For this reason, comparisons of body weight gains can provide a more precise indicator of possible test article toxicity than can comparisons of the raw body weight alone. For this reason, body weight gain, rather than body weight, was selected as the most appropriate criterion for assessment of the possible toxicity of the test and control articles.

Body weight gain calculations were based on animal body weight at the beginning of each week to facilitate analysis of weekly food consumption data and its relationship with body weight gain during the same period.

Overall, the four-week inhalation exposure to aerosols of the test article had no significant dose-, duration-, or frequency-related effect on body weight gain (Tables A-7 to A-10, Appendix A). Statistically significant differences were detected in a number of individual comparisons (see Appendix to Part Two, Statistical Report). However, the overall results of the study provided little evidence to support the hypothesis that increased exposure to the test article (via increased exposure concentration, increased duration of exposure, and/or increased number of exposures) was associated with significant effects on body weight.

Consideration of the patterns of body weight gain observed during the two weekly body weight observations did provide insight into the short-term effects of the test article on body weight. During the exposure period, the first weekly weighing was performed on the fourth exposure day in animals exposed four days per week, and on the second day after the second exposure in animals exposed two days per week. A pattern observed throughout all exposure groups was that body weight gains were small at the first weekly weighing (during or immediately after the exposure period) and were much larger at the second weighing (during the rest period). Because the pattern was observed in both treated and control groups, the effect appears to be related to the general inhalation exposure protocol, rather than to a specific effect of the test article.

In summary, the fractional factorial analysis used for statistical comparisons of body weight gain data did demonstrate a number of individual effects which were statistically significant. However, these differences were small, and often indicated increases, rather than decreases, in body weight gain in groups exposed to the test article. Furthermore, the data demonstrated no pattern suggestive of a dose-response relationship. Because no consistent, dose-related alterations of body weight gains were observed in groups exposed to the test article, it is concluded that the test article had no biologically significant impact on this parameter.



### 2.3 Food Consumption

The statistical approach used to analyze food consumption data also employed multivariate analysis of variance models; polynomial contrasts were used to test for constant effects and linear trends across time. In all cases, data from the two food consumption measurements made per week were summed and averaged to provide an average daily food consumption value for each of 5 weeks. Food consumption data were log transformed prior to analysis.

In summary, all groups exposed to aerosols of the graphite test article or the positive control demonstrated statistically significant decreases in food consumption when compared to filtered air controls (Tables A-11 to A-12, Appendix A). However, in groups exposed to the graphite aerosol, these decreases were not dose-related, and no consistent effect of concentration, duration, or frequency of exposure were observed. These results are in general agreement with the body weight data, in which concentration, duration, and frequency of exposure also had little effect.

### 2.4 Relative Lung Weights

The relative lung weight (lung/body weight ratio) was analyzed statistically using analysis of variance models and log-transformed data. All data were obtained at necropsy, following either the exposure or exposure + recovery periods (Tables A-13 to A-16, Appendix A).

In comparisons of groups exposed to the test article with sex-matched control groups, statistically significant increases in the lung to body weight ratio were observed at the end of the exposure period only in females in the highest exposure group (concentration of 200 mg/m<sup>3</sup>, 4 hours per day, 4 exposures per week). The test article had no effect on lung to body weight ratio in female rats exposed to the test article by other regimens, and had no effect in males in any test article exposure group. By contrast, the lung to body weight ratio was increased in both males and females exposed to the positive control article.

The observation that relative lung weights were increased in female rats receiving the highest total dose of the test article suggests that the test article may be a mild lung irritant. This finding is in general agreement with the results of the pulmonary lavage studies. Because in terms of lung body weight ratios this effect was seen only in females at the highest dose, however, the potency of the test article as a lung irritant in this context appears to be weak.

At the end of the recovery period, significant increases in the lung to body weight ratio were seen in male rats which had been previously exposed to the 200 mg/m<sup>3</sup> of the test article for 4 hours per day, 4 exposures per week and in females exposed at 200 mg/m<sup>3</sup> for either 1

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hour per day, 4 days per week or for 4 hours per day, 2 days per week and in those exposed to 100 mg/m<sup>3</sup> for 4 hours per day, 4 days per week. Significant increases were also seen in both sexes previously exposed to the positive control article.

Statistically significant effects compared with controls were seen at the end of the recovery period in several groups which demonstrated no such effects at the end of treatment. However, because statistical significance was observed only at the end of the recovery period, rather than immediately following the end of exposure, the biological significance of these findings is unknown.

### **3. CLINICAL PATHOLOGY AND HISTOPATHOLOGY**

#### **3.1 Clinical Pathology** Report by B. Levine

Clinical chemistry summary tables for post-exposure and post-recovery tests are shown in Tables A-17 to A-20, Appendix A. Corresponding hematology summary tables are in Tables A-21 to A-24, and corresponding hematology WBC differential summary tables are in Tables A-25 to A-28, Appendix A. Statistical analyses of these data are contained in the Statistical Report in Part Two.

Neither concentration, duration, nor frequency of exposure of the graphite aerosols affected clinical pathology parameters. Sporadic increases and decreases were seen which were not considered biologically significant. For the positive control group, total WBC counts were slightly, but significantly, elevated at the post-exposure timepoint for both sexes. These elevations were primarily due to increased numbers of neutrophils. Recovery from neutrocytosis was not apparent at the post-recovery timepoint. No other clinical pathology measurements were altered for the positive control group.

#### **3.2 Histopathology** Report by M. J. Tomlinson

(See complete Pathology Report included in Appendix B)

### **4. PULMONARY LAVAGE PARAMETERS** Report by R. Sherwood

Results of the cellular counts and differential analysis of the pulmonary lavage study are shown in Table A-29, Appendix A. Results of the alveolar macrophage (AM) phagocytosis assay and pulmonary lavage fluid protein analysis are found in Table A-30, Appendix A. Because of the small sample sizes, the data presented in these tables have been averaged over sex and duration. Statistical analysis of the data indicated that lavage fluid protein increased significantly with

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increased exposure frequency and concentration. Percent mononuclear cells in lavage fluids significantly decreased with increased exposure frequency, duration and concentration. Percent neutrophils in lavage fluids significantly increased with increased exposure frequency, durations and concentration. Total cells and total viable cells recovered by pulmonary lavage were significantly increased with increased exposure duration. In positive control animals, lavage fluid proteins, total cells, total viable cells, percent viable cells, percent mononuclear cells and percent neutrophils remained significantly different from filtered-air controls after a two-week recovery period. In animals exposed to aerosols of the graphite particles, all parameters were within normal values except for total cells and total viable cells after the two-week recovery period. (This observation suggests the use of a three- rather than two-week recovery period for the future studies).

These data indicate that inhalation exposure to graphite particle aerosols did not alter cellular viability or adversely affect fc-mediated phagocytic activity of alveolar macrophages. This suggests the lack of toxicity to cellular functions from the inhaled graphite particle aerosols. Pulmonary lavage of graphite-exposed rats indicated the presence of increased numbers of cells, increased amounts of protein and altered types of pulmonary cells. All of these changes are consistent with the effects of a pulmonary irritant. Under the conditions of the present exposure protocol, no indication of long-term adverse pulmonary effects were noted in these endpoints. These data suggest that analysis of pulmonary lavage fluids for cell numbers and types as well as for the presence of protein provided a sensitive endpoint for determination of pulmonary damage.

## **5. PULMONARY FUNCTION**

Report by J. Tepper

Significant concentration-related changes were obtained from the four pulmonary function tests (flow-volume, pressure-volume, gas dilution and tidal breathing tests) examined in rats exposed to the graphite aerosols compared to the filtered air controls. Overall, the multivariate fractional factorial analysis did not detect any important pulmonary function effects related to the sex of the animals, the duration or frequency of exposure. Furthermore, the analysis revealed that many of the altered pulmonary measurements recovered to within normal limits during the post-recovery period.

### **5.1 Tidal Breathing Test (Ventilation and Breathing Mechanics)**

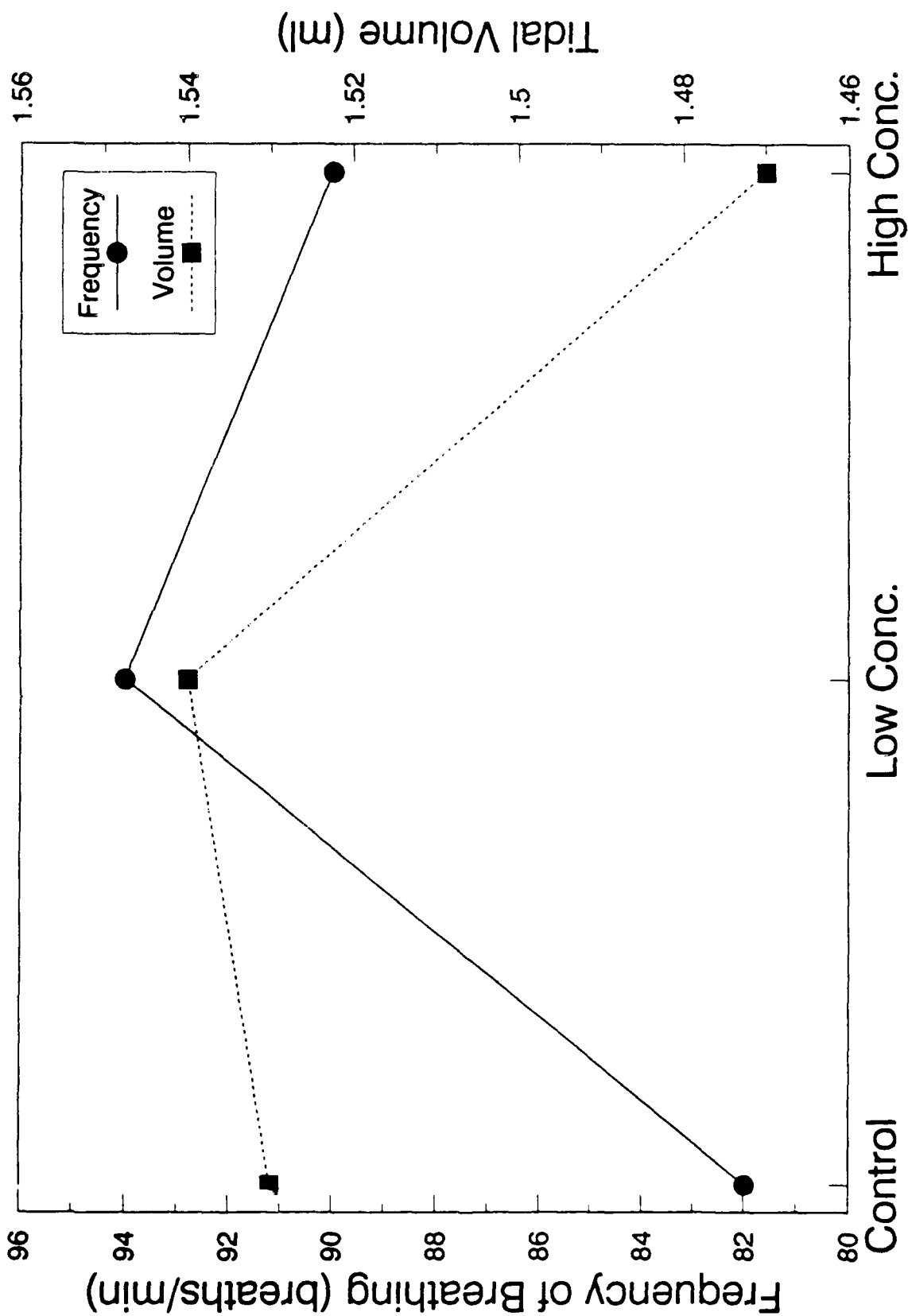
Five of the ten parameters obtained from this test were considered primary measurements (frequency of breathing, tidal volume, esophageal

pressure, lung resistance and dynamic compliance) and thus, were examined with the multivariate analysis. The remaining five measurements (minute ventilation, inspiratory and expiratory times, and inspiratory and expiratory maximal flows) were analyzed in an exploratory data analysis using univariate tests. A significant increase in frequency of breathing ( $P < 0.05$ ) was observed post-exposure (both in the 100 and 200 mg/m<sup>3</sup> groups) when averaged over sex, frequency and duration, and when adjusted for differences in body weight (FIGURE III-1). The increase in frequency was explained primarily by a greater decrease in the time required for expiration than for inspiration. Concomitant with the increase in frequency, a non-significant decrease in tidal volume was observed post-exposure (FIGURE III-1). Dynamic compliance was not significantly effected, but like the chord compliance obtained from the pressure-volume curve (see Figure III-4), there was a decrease with exposure (Table A-31, Appendix A). None of these effects persisted into the post-recovery period. The rats were affected in a somewhat similar manner, but more severely, by the positive control aerosol. Additionally, the increased frequency of breathing and the reduced dynamic compliance did not appear to recover during the post-recovery period.

## 5.2 Gas Dilution Test (Lung Volumes and Diffusion Capacity)

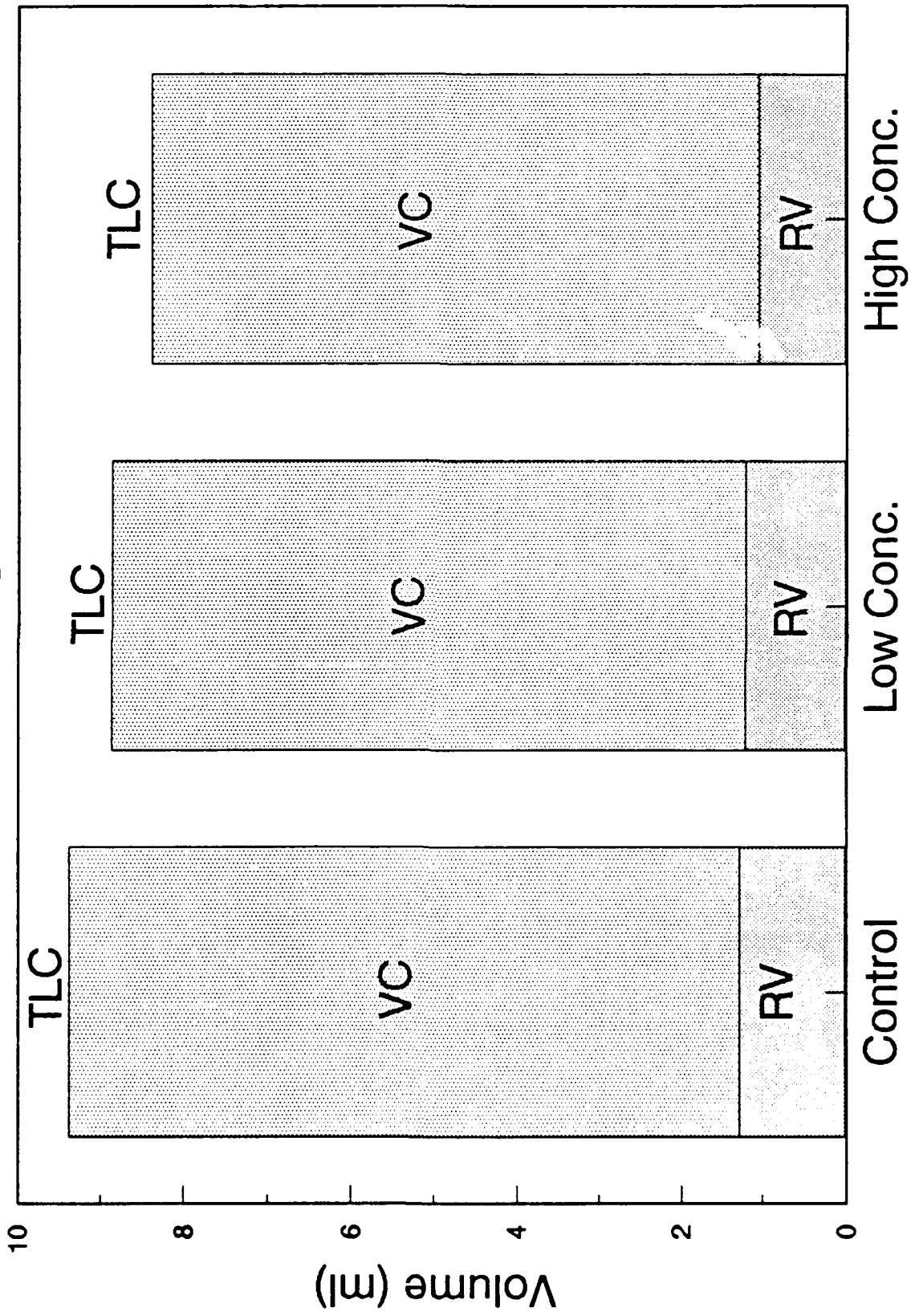
From the gas dilution test, three lung volumes (total lung capacity, vital capacity and residual volume) and the diffusing capacity of carbon monoxide were obtained. Because the diffusing capacity, total lung capacity and vital capacity were primary measures, they were incorporated into the multivariate test. Univariate statistics were used to examine the effects of the test article on residual volume, since residual volume is calculated as total lung capacity minus vital capacity. The analysis revealed that a significant concentration-related decrease in lung volumes occurred post-exposure when averaged over sex, duration and frequency (FIGURE III-2). The diffusing capacity was not significantly affected by exposure, however; a concentration-related decrease in this parameter was also observed (FIGURE III-3). Significant post-recovery decrements were also observed in the 100 mg/m<sup>3</sup> exposure group for both vital capacity and diffusing capacity. Furthermore, a significant concentration by period (post-exposure versus post-recovery) interaction was observed (Table A-32, Appendix A). This interaction indicated that vital capacity and total lung capacity were decreased post-exposure, while post-recovery, both variables were elevated in the 200 mg/m<sup>3</sup> concentration group compared to the 100 mg/m<sup>3</sup> group. Similar reductions in lung volumes and diffusing capacity were also observed for the positive control aerosol.

FIGURE III-1 Ventilation



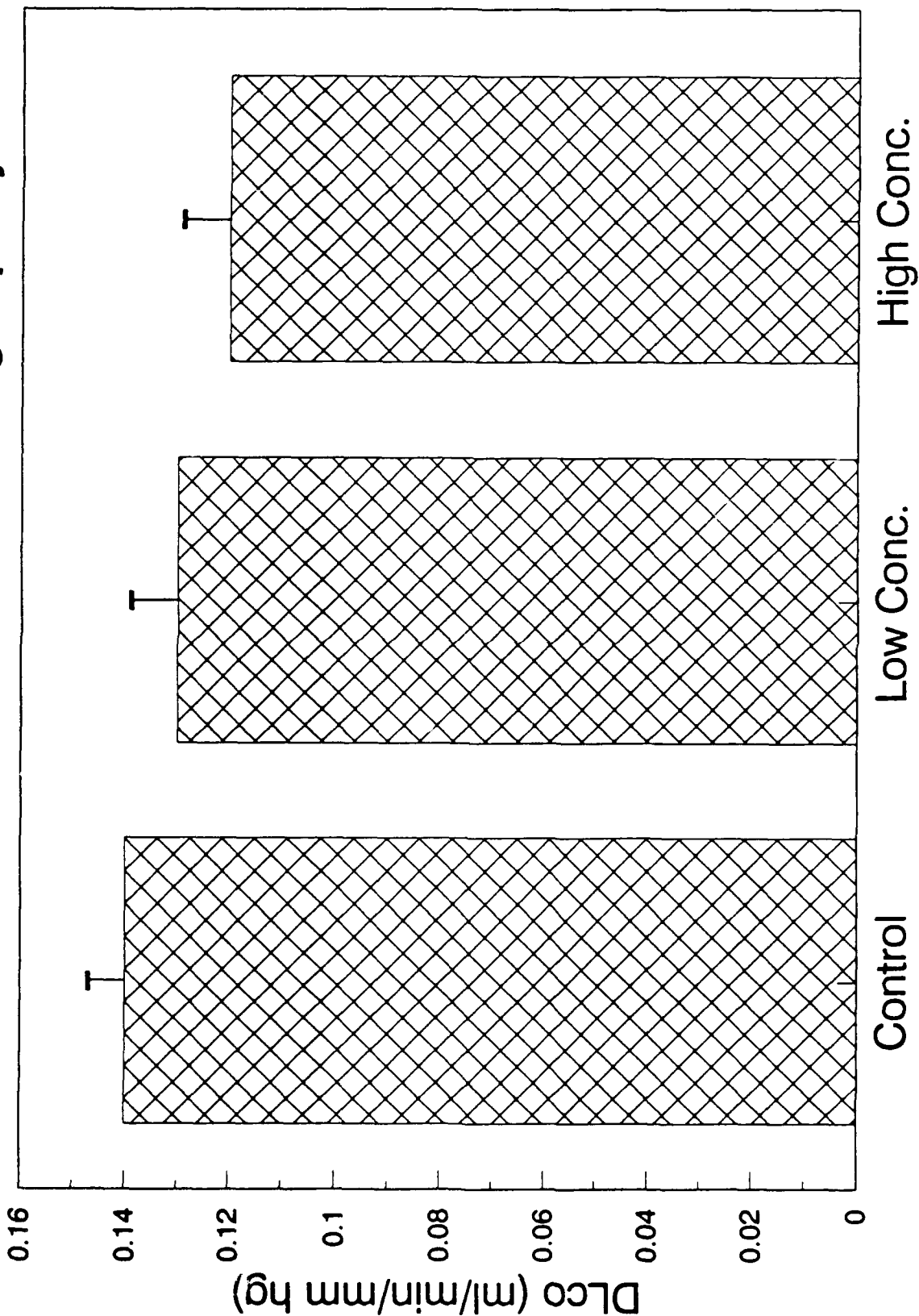
Frequency of breathing was increased while tidal volume was reduced in rats examined immediately post-exposure.

**FIGURE III-2 Lung Volumes**



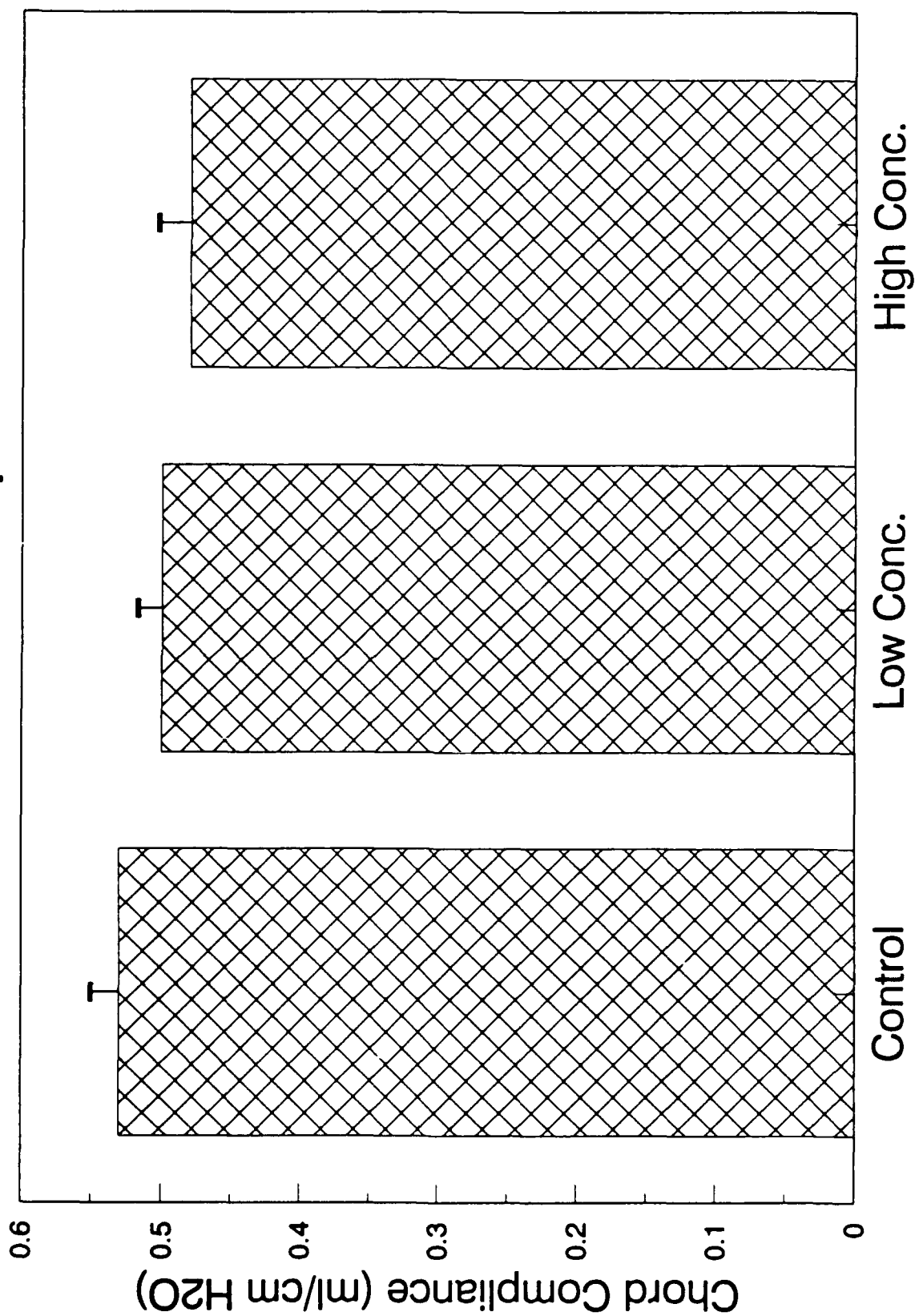
A restrictive lung lesion is indicated by the reduction in total lung capacity, vital capacity and residual volume.

**FIGURE III-3 Carbon Monoxide Diffusing Capacity**



The diffusing capacity of carbon monoxide was reduced in rats examined immediately post-exposure.

**FIGURE III-4 Chord Compliance**



Chord compliance was reduced in rats examined post-exposure, indicating that the lung was stiffer.



### 5.3 Pressure-Volume Test (Compliance Maneuver)

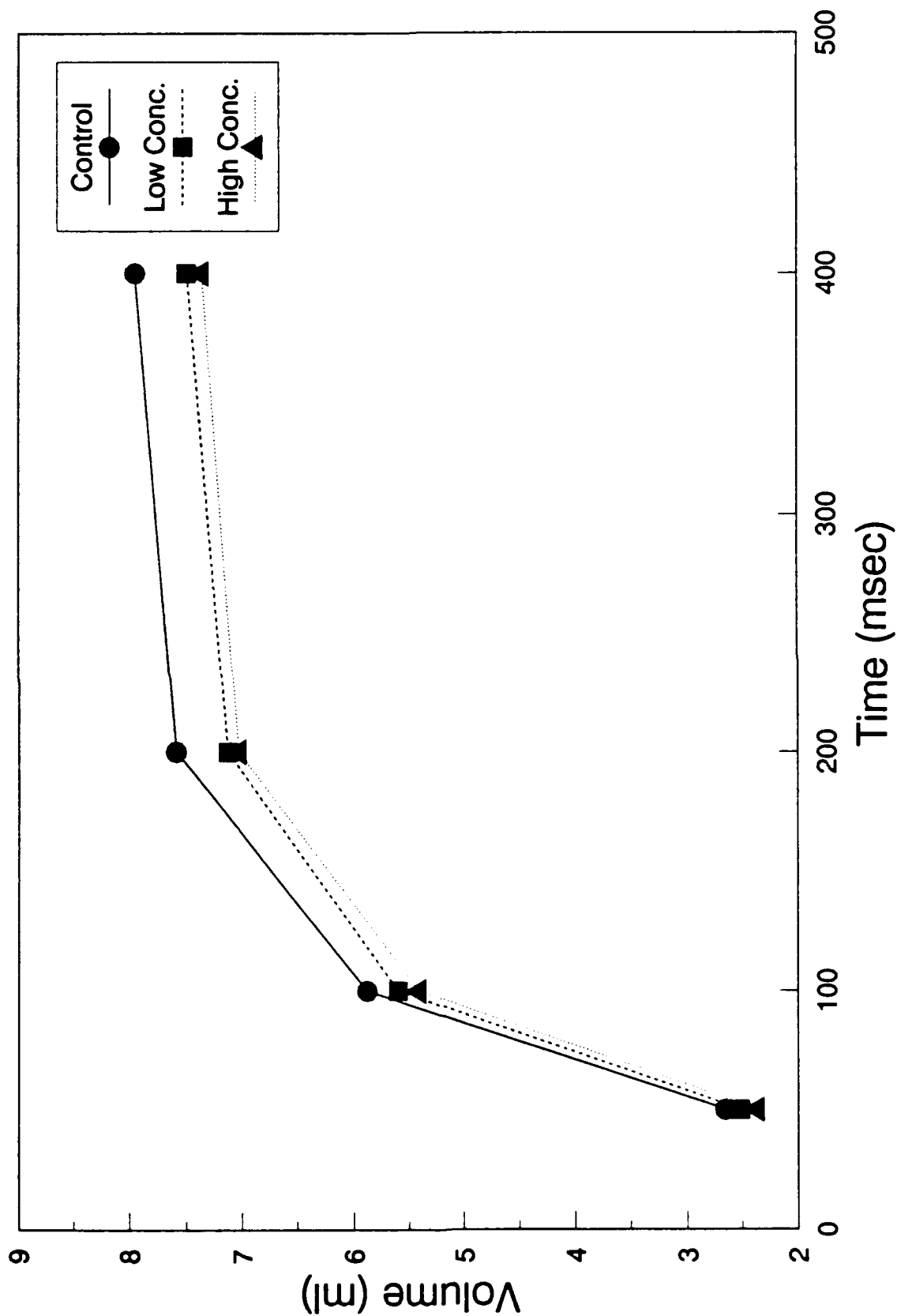
Two measures obtained from the pressure-volume test were evaluated using the multivariate analysis (vital capacity and chord compliance) and one parameter was examined with the exploratory univariate analysis (peak compliance). Neither vital capacity or peak compliance were significantly affected by the treatment. However, the data in FIGURE III-4 suggests that a concentration-related decrease in chord compliance occurred during exposure when averaged over sex, frequency and duration. The positive control aerosol produced pulmonary function changes similar in direction and magnitude to the test aerosol, these effects were also not significant.

A significant duration by frequency interaction was obtained for both vital capacity and chord compliance when averaged over sex, period and concentration. Both measures decreased at the highest exposure frequency (4/wk), when duration increased from 1 hr to 4 hr/day (Table A-33, Appendix A). This effect is consistent with the concentration-related effects. The reverse was true of the low exposure frequency (2/wk).

### 5.4 Flow-Volume Test (Forced Expiratory Maneuver)

Three measurements from this test (forced vital capacity, forced expiratory volume at 100 msec and the forced expiratory flow at 50% of the forced vital capacity) were incorporated into the multivariate analysis as primary variables. Of these variables, a concentration-dependent effect was observed for the forced vital capacity that obtained significance at the 200 mg/m<sup>3</sup> exposure ( $P < 0.05$ ). This effect occurred when averaging over sex, duration and frequency and with adjustment for body weight as a covariate. An exploratory analysis, using eight additional parameters (forced expiratory volumes at 50, 200 and 400 msec, forced expiratory flows at 75 and 25 percent of the remaining forced vital capacity, peak flow, the volume at peak flow and the mid-maximal expiratory flow) obtained from the flow-volume curve, was evaluated using the univariate analysis. Significant effects were obtained for forced expiratory volume at 200 msec ( $P < 0.01$  at both 100 and 200 mg/m<sup>3</sup>) and forced expiratory volume at 400 msec ( $P < 0.5$  at 100 mg/m<sup>3</sup> and  $P < 0.01$  at 200 mg/m<sup>3</sup>) (FIGURE III-5). The forced vital capacity, forced expiratory volume at 200 and 400 msec from the 100 mg/m<sup>3</sup> exposed animals remained significantly depressed after the postrecovery period. No significant effects were observed for any of the flow-derived measurements (peak expiratory flow and forced expiratory flows at 75, 50 and 25% of the forced vital capacity). Furthermore, the positive control aerosol did not alter the characteristics of the flow-volume test.

**FIGURE III-5 Forced Expiratory Volumes**



The volume exhaled during the forced expiratory maneuver was reduced at several time points after the beginning of exhalation in rats examined immediately post-exposure.

A significant sex by duration interaction was observed for the variables forced vital capacity and forced expiratory volume at 100 msec (Table A-34, Appendix A). The interaction indicated that the forced vital capacity and the forced expiratory volume at 100 msec were reduced in male rats exposed 4 hr/day as compared to 1 hr/day, while the reverse was true in female rats (an increase in these volumes at the higher duration). However, it should be kept in mind that these effects occurred when averaged over concentration, period and frequency.

## 5.5 Summary Discussion

Taken collectively, the data suggest the development of a mild restrictive type lesion as evidenced by decreased compliance (chord compliance and dynamic compliance), an impaired capacity to diffuse carbon monoxide and by reduced lung volumes without a reduction in flow. The reduction in lung compliance specifically indicates that more pressure than normal is required to inflate the lung. The reduced diffusing capacity suggests a thickened interstitium. Although the diffusing capacity can be reduced by many factors, the increased neutrophils and protein in the lavage fluid (Tables 29 and 30, Appendix A) would appear to be corroborating evidence of factors that could cause or result in a stiffer lung. The reduction in static lung volumes (total lung capacity, vital capacity and residual volume) as well as the decrease in dynamic measurements of lung volume, obtained from the forced expiratory maneuver, would also point to a restrictive type lesion. Additionally, the resting pattern of breathing was modified such that frequency of breathing was larger, while tidal volume was decreased; an alteration known to increase the efficiency of breathing when the lung is stiff. This is further exemplified by the more prominent decrease in expiratory than inspiratory time, indicating that it is more difficult to inspire, but that lung expiration is hastened due to increased elastic recoil (stiffer lung).

The positive control aerosol produced similar effects in direction and magnitude as the test aerosol. Tidal breathing measurements, and the reduction in diffusing capacity and lung volumes were significant. However, the pressure-volume and flow-volume tests were either not changed or only minimally.

All of these data obtained for the test aerosol should be interpreted with caution because the data were averaged over sex, frequency and duration and because many of the aforementioned effects were small or not statistically significant. Additionally, many of the effects were significant only after adjustment for differences in body weight. This was not particularly surprising since male and female animals with large difference in body weight, and therefore lung volumes, were combined for the analysis. Since few interactions occurred, this suggests either that the effects were not consistent or that the variability inherent in the small sample made it difficult to detect such interactions. Thus, this study failed to detect any significant effects of the factors sex, frequency and duration.

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#### IV. CONCLUSIONS AND RECOMMENDATIONS

The objective of this four-week inhalation toxicity study to aerosols of graphite particles in male and female F344/N rats was to evaluate the effects of exposure concentration, daily duration, weekly frequency and sex of the animals on selected biologic endpoint parameters including pulmonary lavage, pulmonary function, lung weights, histopathology and clinical pathology. Endpoints were evaluated after the last exposure and after a two-week recovery period. In addition, all animals were monitored throughout the study for clinical signs, body weights, and selected groups for food consumption. A fractional factorial design, which allows for the most efficient use of the experimental resources, and multivariate analysis of variance models were used for the statistical evaluation of these studies.

The results of the statistical computations revealed a remarkably striking absence of significant main effects of any of these factors, suggesting that differences in levels of concentration, frequency, and duration are unrelated to outcome. A notable exception, however, was the pulmonary lavage parameters. Here, several measurements were affected by all three factors, either significantly increased or decreased.

There were no mortalities and clinical observations provided no evidence of significant treatment-related toxicity in any experimental group throughout the study. Overall, the four-week inhalation exposure to the graphite aerosols had no significant dose-, duration-, or frequency-related effect on body weight gain, food consumption and clinical pathology parameters.

Although the overall effects of the graphite aerosol exposures on relative lung weights are inconsistent and are difficult to interpret biologically, those effects that were found were in some of the high concentration/frequency/duration groups - a condition we will explore further in the Phase III study (see below).

Pulmonary lavage of graphite aerosol-exposed rats indicated the presence of increased numbers of cells, increased amounts of protein and altered types of pulmonary cells. Statistical analysis revealed that these data were affected in various ways by exposure duration, frequency and concentration, respectively. All of these changes are consistent with the effects of a pulmonary irritant.

Significant concentration-related changes were obtained from the four pulmonary function tests (flow-volume, pressure-volume, gas dilution and tidal breathing tests) examined in rats exposed to the graphite aerosols compared to the filtered air controls. Overall, the study did not detect any important pulmonary function effects related to the sex of the animals, or the duration and frequency of exposure.

Based on these overall results of Phase II, our recommendation is to use the "worst case" conditions for the Phase III studies, i.e., 200 mg/m<sup>3</sup> of the graphite aerosol in exposures of 4-hr daily durations at frequencies of 4 exposures per week. We are also recommending a 3-,

instead of a 2-week recovery period due to some remaining effects in lavage parameters after the 2-week recovery. Since sex did not have a decisive role in the Phase II studies, we recommend to conduct the Phase III studies with male rats only.

As described in our proposal in response to the RFP, the experimental design of the 4-week Phase III studies includes six groups: aerosols of one material, fog oil, will be tested at two concentrations (1000 and 500 mg/m<sup>3</sup>, as per instructions received earlier from the Government) in two groups each. Aerosols of the second material, graphite particles will be tested at a single concentration (200 mg/m<sup>3</sup>, based on the Phase II study results) in three of the six groups, in one as the only test article and in two in combination with each of the two concentrations of the fog oil. The sixth group will consist of filtered air controls.

In terms of the experimental endpoints, according to the proposal we have mandated core study endpoints (body weights, food consumption, clinical signs, histopathology, in vivo and in vitro xenobiotic metabolism studies), and "optional" endpoints that could be selected based on the Phase II results. We have requested earlier to delete the in vivo xenobiotic metabolism assays from the core study. In terms of the optional endpoints, we are recommending in the first place to conduct the lavage studies, but with omission of the phagocytosis assay where we found no effects, and instead conduct an in vivo pulmonary bactericidal activity study on a separate group of rats. This bactericidal activity assay has proven to be very sensitive in our hands in evaluating effects of aerosol exposures, among them affects of exposures to red phosphorus/butyl rubber smokes. (C. Aranyi, M. C. Henry, S. C. Vana, R. D. Gibbons, and W. O. Iverson: Effects of Multiple Intermittent Inhalation Exposures to Red Phosphorus/Butyl Rubber Obscurant Smokes in Sprague-Dawley Rats; Inhal. Tox. Premier Issue; 65-78, 1988. Also see our Final Report ADA-189254 on Contract No. DAMD-82-C-2121 submitted in 1987 to the U.S. Army Medical Research and Development Command.)

Our second endpoint choice is pulmonary function. Ultimately, it is dysfunction, not pathology that determines the health status of the animal. Measurement of pulmonary function in animals with correlative structural analysis can be used to provide direct evidence of the potential functional disabilities that might be seen in man. Additionally, such tests are useful in discerning effects where pathological changes may not be easily detectable. In addition, we would like to see the effects of the fog oil/graphite particle aerosol mixtures on pulmonary physiology in this Phase III 4-week study and use the results as a basis for considering to monitor the effects of the 13-week exposures by this endpoint in Phase IV. (The animals for pulmonary lavage and pulmonary function will be shared, and we will need a separate group for the pulmonary bactericidal activity assay).

The conduct of clinical pathology is our last optional choice, but basically we feel that it should be done in spite of not having found effects in Phase II. We all know that the biggest expense in an inhalation toxicology study is to conduct the exposures, and the more endpoint information we can obtain, the more efficiently our funds have been used.

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## V. QUALITY ASSURANCE STATEMENT

Study Title: Four-Week Repeated Dose Inhalation Toxicity Study with Aerosols of a Solid Particulate Test Material in Male and Female F344/N Rats to Evaluate the Effects of Exposure Concentration, Duration, Frequency, and Recovery Time on Various Biological Endpoints.

Project Number: L06234

Study Number: 1

Study Director: Jeannie Bradof

Report Audit Dates: February 5 - 7, 11 - 15, 18 - 22, 25, 27; March 22 - 29; and April 1, 1991

This study has been subjected to inspections and the report has been audited by the IITRI Quality Assurance Unit. The report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study. There were no significant deviations from the EPA Good Laboratory Practice Standards (40 CFR Part 792).

The following are the inspection dates and the dates inspection reports were submitted:

<u>Dates of Inspection</u>	<u>Inspection Reports Submitted to:</u> <u>Study Director</u>	<u>Management</u>
9/20/90	9/20/90	9/20/90
9/28/90	9/28/90	9/28/90
10/1/90	10/1/90	10/1/90
10/25/90	10/25/90	10/25/90
11/1/90	11/1/90	11/1/90
11/2/90	11/2/90	11/2/90
11/16/90	11/16/90	11/16/90

\_\_\_\_\_  
Ronald A. Boyne, B.S.  
Manager, Quality Assurance

\_\_\_\_\_  
Date

**PART ONE**

**APPENDIX A: TABLES**

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## INTRODUCTORY COMMENTS

Most tables presented here were selected from the appropriate sections of the Part Two Statistical Report Appendix. In general, the tables selected presented data for all parameters measured and for all exposure groups and sample sizes defined in the experimental design. For presentation in this Appendix, where all tables associated with the experimental "Results" section are displayed, we added a short descriptive title and the appropriate footnotes and abbreviations to each table.

The following explanations to the tables are provided to aid the reader in understanding some of the changes indicated in the footnotes. In addition, the specific abbreviations added to the footnotes were also incorporated to aid the reviewer in identifying the complex endpoint/experimental design features presented in the tables.

Changes in samples sizes: Sample sizes for some means presented in these tables are different from the sample sizes specified in the protocol. In body weight gain and food consumption data, differences were the result of weights which were missed in a few isolated cases or which resulted in weight gain or food consumption values which differed from the group means by more than two standard deviations, or were outside of the expected values for animals of that age and sex.

In the case of hematology or white cell differential data, these differences are the result of clotted samples which could not be analyzed, or missing specimens. For the clinical chemistry data, the differences are due to the fact that no specimens were collected for two rats, and that one SDH value was excluded due to being more than three standard deviations from the mean.

Changes in group means: Due to late discovery of some small errors in the records, a few corrections (9 out of the total 2016 values analyzed for the 144 post-exposure assay animals) were made to the post-exposure clinical chemistry data after the statistical analysis had been completed. This resulted in minor changes in the affected group means shown in these tables relative to the corresponding values in the Appendix Tables of the Statistical Report of Part II. (Because of the magnitude of the statistical analysis effort, it could not be repeated with the corrected values). Of these nine values changed, two were for PHOS data, one each were for TRIG and ALT data, and five were for SDH data (no more than two were in any exposure group).

Similarly, one body weight value (out of the approximately 3360 values incorporated in the exposure period body weight gain analysis) was corrected after the statistical analysis and caused minor changes to the means in two weight gain periods as shown in these tables relative to the corresponding values shown in the Appendix of the Statistical Report.

Careful review of each of these changes in the means resulted in the judgement that these differences did not impact on the overall evaluation of the study data for any of the specific endpoints affected.

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APPENDIX A

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TABLE A-1

DAILY GRAPHITE AEROSOL MASS CONCENTRATIONS AT THE 100 mg/m<sup>3</sup> TARGET  
LEVEL DURING THE 4-WEEK EXPOSURES: CHAMBER NO.1 --EXPOSURE DURATION OF  
ONE HOUR WITH FREQUENCIES OF 4 AND 2 DAYS/WEEK

EXPOSURE DATE	CONC. mg/m <sup>3</sup>	N	% MEAN/TARGET
10/02/90	97.5	1	97.5
10/03/90 *	98.1	1	98.1
10/04/90 *	95.5	1	95.5
10/05/90	81.3	1	81.3
10/08/90	87.3	1	87.3
10/09/90	88.9	1	88.9
10/10/90 *	96.1	1	96.1
10/11/90 *	92.4	1	92.4
10/12/90	92.9	1	92.9
10/15/90	88.2	1	88.2
10/16/90	84.7	1	84.7
10/17/90 *	95.6	1	95.6
10/18/90 *	106.0	1	106.0
10/19/90	101.0	1	101.0
10/22/90	89.7	1	89.7
10/23/90 *	81.5	2	81.5
10/24/90 *	93.6	1	93.6
10/25/90	86.4	1	86.4
10/29/90	107.0	1	107.0
10/30/90 *	114.0	1	114.0
10/31/90 *	102.0	1	102.0
11/01/90 *	96.5	1	96.5

## STUDY SUMMARY

	4 DAYS/WK. =====	2 DAYS/WK. (* DAYS) =====
MEAN	94.4	97.4
STD.DEV	8.3	8.2
N	22	11
MINIMUM	81.3	81.5
MAXIMUM	114	114

TABLE A-2

DAILY GRAPHITE AEROSOL MASS CONCENTRATIONS AT THE 100 mg/m<sup>3</sup> TARGET  
LEVEL DURING THE 4-WEEK EXPOSURES: CHAMBER NO.5 --EXPOSURE DURATION OF  
FOUR HOURS WITH FREQUENCIES OF 4 AND 2 DAYS/WEEK

EXPOSURE DATE	CONC. mg/m <sup>3</sup>	SD	N	%RSD	MIN. mg/m <sup>3</sup>	MAX. mg/m <sup>3</sup>	% MEAN/TARGET
10/09/90	115.0	7.89	4	6.86	108.0	126.0	115.0
10/10/90 *	106.0	16.30	4	15.40	92.6	128.0	106.0
10/11/90 *	99.0	9.16	4	9.25	90.2	111.0	99.0
10/12/90 *	100.0	7.87	4	7.87	95.0	112.0	100.0
10/16/90	104.0	5.76	4	5.54	96.9	110.0	104.0
10/17/90 *	97.2	8.71	4	8.96	85.9	105.0	97.2
10/18/90 *	92.4	5.35	4	5.79	88.4	100.0	92.4
10/19/90 *	94.4	7.12	4	7.54	87.0	103.0	94.4
10/23/90	85.0	3.61	4	4.25	79.8	88.1	85.0
10/24/90 *	88.4	4.60	4	5.20	85.1	95.0	88.4
10/25/90 *	88.0	8.99	4	10.20	75.5	95.8	88.0
10/26/90 *	89.9	3.98	4	4.43	84.5	92.9	89.9
10/29/90	93.8	3.89	4	4.15	89.7	98.8	93.8
10/30/90 *	98.2	4.08	4	4.15	94.1	103.0	98.2
10/31/90 *	103.0	4.72	4	4.58	96.6	108.0	103.0
11/01/90 *	101.0	5.51	4	5.46	95.1	108.0	101.0
11/02/90 *	108.0	6.56	4	6.07	98.6	113.0	108.0
11/03/90 *	104.0	4.68	4	4.50	99.9	110.0	104.0

## STUDY SUMMARY

	4 DAYS/WK. =====	2 DAYS/WK. (* DAYS) =====
MEAN	98.2	97.8
STD.DEV	7.9	6.5
N	18	14
MINIMUM	85	88
MAXIMUM	115	108

TABLE A-3

DAILY GRAPHITE AEROSOL MASS CONCENTRATIONS AT THE 200 mg/m<sup>3</sup> TARGET  
LEVEL DURING THE 4-WEEK EXPOSURES: CHAMBER NO.2 --EXPOSURE DURATION OF  
ONE HOUR WITH FREQUENCIES OF 4 AND 2 DAYS/WEEK

EXPOSURE DATE	CONC. mg/m <sup>3</sup>	N	% MEAN/TARGET
10/08/90	182	1	91.0
10/09/90	193	1	96.5
10/10/90	177	1	88.5
10/11/90 *	203	1	101.5
10/12/90 *	208	1	104.0
10/15/90	215	1	108.0
10/16/90	200	1	100.0
10/17/90	233	1	116.5
10/18/90 *	195	1	97.5
10/19/90 *	189	1	94.5
10/22/90	169	1	84.5
10/23/90	221	1	110.5
10/24/90	217	1	108.5
10/25/90 *	183	1	91.5
10/26/90 *	202	1	101.0
10/28/90	197	1	98.5
10/29/90	197	1	98.5
10/30/90	194	1	97.0
10/31/90 *	190	1	95.0
11/01/90 *	213	1	106.5
11/02/90 *	197	1	98.5
11/03/90 *	182	2	91.0

## STUDY SUMMARY

	4 DAYS/WK. =====	2 DAYS/WK. (* DAYS) =====
MEAN	198.0	196
STD.DEV	15.4	10.4
N	22	10
MINIMUM	169	182
MAXIMUM	233	213

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TABLE A-4

DAILY GRAPHITE AEROSOL MASS CONCENTRATIONS AT THE 200 mg/m<sup>3</sup> TARGET  
LEVEL DURING THE 4-WEEK EXPOSURES: CHAMBER NO.3 --EXPOSURE DURATION OF  
FOUR HOURS WITH FREQUENCIES OF 4 AND 2 DAYS/WEEK

EXPOSURE DATE	CONC. mg/m <sup>3</sup>	SD	N	%RSD	MIN. mg/m <sup>3</sup>	MAX. mg/m <sup>3</sup>	% MEAN/TARGET
10/01/90	191	9.49	4	4.97	177	198	95.5
10/02/90	198	7.94	4	4.01	192	210	99.0
10/03/90	186	6.65	4	3.57	176	191	93.0
10/04/90 *	193	6.29	4	3.26	186	199	96.5
10/05/90 *	196	10.60	4	5.41	184	208	98.0
10/08/90	204	6.14	4	3.01	196	211	102.0
10/09/90	244	39.30	7	16.10	198	291	122.0
10/10/90 *	200	7.32	4	3.66	190	207	100.0
10/11/90 *	189	4.11	4	2.17	184	194	94.5
10.15/90	204	6.58	4	3.52	196	212	102.0
10/16/90	192	8.06	4	4.20	181	200	96.0
10/17/90 *	200	5.12	4	2.56	194	205	100.0
10/18/90 *	197	5.68	4	2.88	189	202	98.5
10/19/90 *	202	4.20	4	2.08	197	207	101.0
10/21/90	204	6.32	4	3.10	196	210	102.0
10/22/90	195	14.30	4	7.33	182	214	97.5
10/23/90	186	6.55	4	3.52	178	192	93.0
10/24/90 *	187	8.52	4	4.56	180	199	93.5
10/25/90 *	190	4.50	4	2.37	186	196	95.0
10/28/90	201	20.10	4	10.00	184	230	100.5
10/29/90	190	4.79	4	2.52	185	196	95.0
10/30/90 *	195	4.86	4	2.49	192	202	97.5
10/31/90 *	198	11.60	4	5.86	187	212	99.0
11/01/90 *	190	6.24	4	3.28	184	198	95.0

## STUDY SUMMARY

	4 DAYS/WK. =====	2 DAYS/WK. (* DAYS) =====
MEAN	197.2	195
STD.DEV	11.5	4.9
N	24	12
MINIMUM	186	187
MAXIMUM	244	202

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TABLE A-5

DAILY GRAPHITE AEROSOL MASS CONCENTRATIONS AT THE 200 mg/m<sup>3</sup> TARGET  
LEVEL DURING THE 4-WEEK EXPOSURES: CHAMBER NO.4 --EXPOSURE DURATION OF  
FOUR HOURS WITH A FREQUENCY OF 4 DAYS/WEEK

EXPOSURE DATE	CONC. mg/m <sup>3</sup>	SD	N	%RSD	MIN. mg/m <sup>3</sup>	MAX. mg/m <sup>3</sup>	% MEAN/TARGET
10/01/90	172	5.97	4	3.47	164	178	86.0
10/02/90	153	9.93	5	6.49	142	167	76.5
10/03/90	204	10.20	4	5.00	191	216	102.0
10/04/90	180	15.10	4	8.39	160	193	90.0
10/05/90	210	8.58	4	4.07	201	220	105.0
10/08/90	175	3.30	4	1.88	173	180	87.5
10/09/90	159	14.90	6	9.37	136	172	79.5
10/10/90	187	18.30	4	9.79	166	206	93.5
10/11/90	181	5.10	4	2.80	174	186	90.5
10/12/90	220	11.10	4	5.04	212	236	110.0
10/13/90	192	7.19	4	3.74	181	197	96.0
10/16/90	191	22.70	6	11.90	148	207	95.5
10/17/90	214	8.44	4	3.94	206	225	107.0
10/18/90	218	11.40	4	5.23	201	225	109.0
10/19/90	222	16.20	4	7.30	208	241	111.0
10/21/90	189	6.13	4	3.24	185	198	94.5
10/22/90	196	18.60	4	9.49	178	213	98.0
10/23/90	189	16.70	4	8.84	167	204	94.5
10/24/90	203	12.70	4	6.26	190	220	101.5
10/25/90	190	11.00	4	5.79	175	200	95.0
10/26/90	212	6.98	4	3.29	204	220	106.0
10/28/90	201	21.30	4	10.60	170	218	100.5
10/29/90	207	17.00	4	8.21	188	222	103.5
10/30/90	207	5.60	4	2.70	200	212	103.5
10/31/90	208	11.70	4	5.62	193	221	104.0
11/01/90	199	11.10	4	5.58	188	214	99.5
11/02/90	202	7.50	4	3.71	194	210	101.0
11/03/90	194	9.33	4	4.81	185	206	97.0

## STUDY SUMMARY

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MEAN	195.5
STD.DEV	17.3
N	28
MINIMUM	153
MAXIMUM	222



TABLE A-6

## CLINICAL SIGNS RECORDED IN MALE AND FEMALE RATS DURING FOUR-WEEK EXPOSURE AND TWO-WEEK RECOVERY PERIODS

Group <sup>a</sup>	Sex	Observation	Exposure Period						Recovery Period					
			Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
			Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2
I	M	No signs observed	80/80 <sup>b</sup>	80/80	80/80	80/80	80/80	80/80	80/80	80/80	37/40	31/40	36/40	34/36c
		Red fur around nose									3/40	8/40	4/40	2/36c
		Red nasal discharge										1/40		
II	F	No signs observed	80/80	79/80	79/80	79/80	79/80	79/80	79/80	79/80	37/40	36/40	31/40	35/36c
		Redness around eye(s)	1/80	1/80	1/80	1/80	1/80	1/80	1/80	1/80	1/40	1/40	1/40	1/36c
		Missing eye		1/80	1/80	1/80	1/80	1/80	1/80	1/80	1/40	1/40	1/40	1/36c
		Red fur around nose									2/40	3/40	7/40	
		Red nasal discharge										1/40		
		No signs observed	15/15	0/15	15/15	0/15	13/15	0/15	12/15	0/15	*	*	*	*
		Red fur around nose					2/15	2/15	3/15		*	*	*	*
		Discolored paws		6/15							*	*	*	*
		Black fur around nose		12/15					3/15		*	*	*	*
F		Test article on fur	15/15	15/15	15/15	0/15	15/15	15/15	15/15	15/15	*	*	*	*
		No signs observed	15/15	0/15	15/15	0/15	15/15	0/15	12/15	0/15	15/15	12/15	7/15	3/15
		Red fur around nose												
		Red nasal discharge												
		Black fur around nose		12/15										
		Test article on fur	15/15	15/15	15/15	0/15	15/15	15/15	3/15	15/15	3/15	8/15	12/15	2/15

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TABLE A-6  
(Continued)

CLINICAL SIGNS RECORDED IN MALE AND FEMALE RATS DURING FOUR-WEEK EXPOSURE AND TWO-WEEK RECOVERY PERIODS

Group <sup>a</sup>	Sex	Observation	Exposure Period						Recovery Period					
			Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
			Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2
III	M	No signs observed	15/15 <sup>b</sup>	0/15	3/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	2/15	0/15
		Test article on fur		15/15	12/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15	11/15	6/15
		Red fur around nose					1/15				3/15	2/15	8/15	10/15
		Red nasal discharge												2/15
IV	F	No signs observed	15/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	*	*	*	*
		Test article on fur		15/15	15/15	15/15	15/15	15/15	15/15	15/15	*	*	*	*
		Red fur around nose												
V	M	No signs observed	15/15	0/15	15/15	0/15	15/15	0/15	15/15	0/15	*	*	*	*
		Test article on fur		15/15	15/15	15/15	15/15	15/15	15/15	15/15	*	*	*	*
		Diarrhea			1/15						*	*	*	*
VI	F	No signs observed	15/15	0/15	3/15	0/15	12/15	0/15	0/15	0/15	0/15	0/15	0/15	4/12c
		Test article on fur		15/15	12/15	15/15	3/15	15/15	15/15	15/15	15/15	12/15	11/15	5/12c
		Red fur around nose									4/15	7/15	6/15	3/12c

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TABLE A-6  
(Continued)

CLINICAL SIGNS RECORDED IN MALE AND FEMALE RATS DURING FOUR-WEEK EXPOSURE AND TWO-WEEK RECOVERY PERIODS

Group <sup>a</sup>	Sex	Observation	Exposure Period				Recovery Period			
			Week 1		Week 2		Week 3		Week 4	
			Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2
VI	M	No signs observed	15/15 <sup>b</sup>	0/15	12/15	0/15	0/15	0/15	0/15	0/15
		Test article on fur		15/15	3/15	15/15	15/15	15/15	15/15	15/15
		Red fur around nose							11/15	11/15
		Red nasal discharge							4/15	2/15
VII	F	No signs observed	15/15	0/15	12/15	0/15	0/15	0/15	*	*
		Test article on fur		15/15	3/15	15/15	15/15	15/15	*	*
		Red fur around nose					12/15		*	*
										*
	M	No signs observed	15/15	0/15	3/15	0/15	0/15	0/15	*	*
		Test article on fur		15/15	12/15	15/15	15/15	15/15	*	*
										*
										*
	F	No signs observed	15/15	0/15	3/15	0/15	0/15	0/15	0/15	0/15
		Test article on fur		15/15	12/15	15/15	15/15	15/15	15/15	15/15
		Red fur around nose					5/15		1/15	1/15
		Red nasal discharge					3/15			1/15
VIII	M	No signs observed	15/15	0/15	0/15	0/15	0/15	0/15	*	*
		Test article on fur		15/15	15/15	15/15	15/15	15/15	*	*
		Red fur around nose				2/15	1/15	1/15	*	*
		Red nasal discharge				1/15			*	*
	F	No signs observed	15/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15
		Test article on fur		15/15	15/15	15/15	15/15	15/15	15/15	15/15
		Red fur around nose								
		Red nasal discharge								

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TABLE A-6  
(Continued)

CLINICAL SIGNS RECORDED IN MALE AND FEMALE RATS DURING FOUR-WEEK EXPOSURE AND TWO-WEEK RECOVERY PERIODS

Group <sup>a</sup> Sex	Observation	Exposure Period						Recovery Period					
		Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
		Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2	Obs.#1	Obs.#2
IX M	No signs observed	15/15 <sup>b</sup>	0/15	0/15	0/15	1/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	Test article on fur	15/15	15/15	15/15	15/15	14/15	15/15	15/15	15/15	15/15	15/15	15/15	13/15
	Red fur around nose									1/15	1/15	2/15	3/15
F	No signs observed	15/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	*	*	*	*
	Test article on fur	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15	*	*	*	*
X M	No signs observed	40/40	20/40	24/40	12/40	10/40	4/40	4/40	0/40	0/20	0/20	0/20	0/18c
	Test article on fur		20/40		20/40		8/40		19/40				
	Red fur around nose			16/40	19/40	30/40	33/40	36/40	40/40	20/20	20/20	20/20	18/18c
	Red nasal discharge				1/40	1/40	1/40			3/20	3/20	6/20	2/18c
F	No signs observed	40/40	20/40	26/40	11/40	9/40	3/40	5/40	0/40	0/16c	0/20	0/20	1/18c
	Test article on fur		20/40		20/40		8/40		12/40				
	Red fur around nose			12/40	17/40	31/40	31/40	35/40	40/40	16/16c	20/20	20/20	17/18c
	Red nasal discharge			3/40	1/40	9/40	1/40	2/40	2/40	1/16c	2/20	5/20	3/18c
	Hunched posture			1/40	1/40	1/40							

<sup>a</sup> Exposure Classes II (C1D1F1), III (C2D1F1), IV (C1D1F2), V (C2D1F2), VI (C1D2F1), VII (C2D2F1), VIII (C1D2F2), and IX (C2D2F2) received aerosols of the test article, Powder A1, where C1 and C2 were 100 and 200 mg/m<sup>3</sup>, D1 and D2 were one and four hr, and F1 and F2 were two and four exposures per week, respectively. Exposure Class I (C0D2F2) was filtered air control and Exposure Class X (C0D2F2) received aerosols of positive control particles at 200 mg/m<sup>3</sup>.

<sup>b</sup> Incidence of observation/number of animals observed.

<sup>c</sup> One cage of rats was omitted from observation at this period.

\* All animals were sacrificed at the end of the exposure period.

**TABLE A-7**  
**SUMMARY OF BODY WEIGHT GAINS OF MALE RATS**  
**EXPOSURE PERIOD**

GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
Control	4 / week	4 Hr/Day							
		mean	8.25	12.86	6.40	12.09	6.30	10.79	6.82
		sd	2.63	3.76	3.04	3.47	3.06	3.94	3.86
		n	79 <sup>a</sup>	80	80	80	80	80	80
LowConc	2 / week	1 Hr/Day							
		mean	7.20	16.47	2.07	15.80	3.87	13.93	.
		sd	3.17	1.85	2.71	2.83	2.61	2.89	. <sup>b</sup>
		n	15	15	15	15	15	15	0
		4 Hr/Day							
		mean	1.93	15.40	2.60	14.07	.13	12.07	3.80
		sd	2.60	1.50	2.26	3.65	1.88	2.58	2.70
		n	15	15	15	15	15	15	15
	4 / week	1 Hr/Day							
		mean	7.07	16.67	4.33	12.20	6.07	10.13	5.60
		sd	1.98	3.37	3.75	4.02	2.28	2.42	2.53
		n	15	15	15	15	15	15	15
		4 Hr/Day							
		mean	1.53	16.40	3.27	10.47	6.67	3.87	-4.53
		sd	8.77	6.79	3.83	3.42	3.58	9.37	16.84
		n	15	15	15	15	15	15	15

TABLE A-7  
(Continued)

SUMMARY OF BODY WEIGHT GAINS OF MALE RATS  
EXPOSURE PERIOD

GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
HighConc 2 / week 1 Hr/Day									
		mean	.87	15.73	2.53	12.27	1.80	10.60	5.47
		sd	3.16	6.73	3.62	3.28	3.12	3.09	2.45
		n	15	15	15	15	15	15	15
4 Hr/Day									
		mean	8.73	14.27	-.87	16.20	2.67	13.53	.
		sd	3.20	3.45	3.64	3.69	2.41	2.64	.
		n	15	15	15	15	15	15	0 <sup>b</sup>
4 / week 1 Hr/Day									
		mean	5.67	13.27	6.33	9.47	6.40	7.73	7.67
		sd	3.33	3.75	2.29	3.07	2.44	3.13	3.46
		n	15	15	15	15	15	15	15
4 Hr/Day									
		mean	6.47	14.93	6.07	12.00	5.93	8.67	4.93
		sd	1.96	2.37	1.91	3.61	2.55	2.55	2.79
		n	15	15	15	15	15	15	15
PosCont 4 / week 4 Hr/Day									
		mean	6.15	13.38	4.56 <sup>c</sup>	9.49 <sup>c</sup>	5.92	7.30	6.68
		sd	7.51	3.14	3.79 <sup>d</sup>	4.98 <sup>d</sup>	2.85	5.66	4.65
		n	40	40	39	39	40	40	40

<sup>a</sup> One animal outside expected weight range excluded.

<sup>b</sup> All male rats of this exposure group were included in post-exposure assays and did not have a full three-day observation period.

<sup>c</sup> Corrected mean (see explanation for tables).

<sup>d</sup> One animal missed at one weighing, affecting two weight gain periods.

ABBREVIATIONS

BWT	-	Body Weight
3DAYWK1A	-	Week 1 Period 1 (BWT gain for 3 day period in grams)
4DAYWK1B	-	Week 1 Period 2 (BWT gain for 4 day period in grams)
3DAYWK2A	-	Week 2 Period 1 (BWT gain for 3 day period in grams)
4DAYWK2B	-	Week 2 Period 2 (BWT gain for 4 day period in grams)
3DAYWK3A	-	Week 3 Period 1 (BWT gain for 3 day period in grams)
4DAYWK3B	-	Week 3 Period 2 (BWT gain for 4 day period in grams)
3DAYWK4A	-	Week 4 Period 1 (BWT gain for 3 day period in grams)

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**TABLE A-8**  
**SUMMARY OF BODY WEIGHT GAINS OF FEMALE RATS**  
**EXPOSURE PERIOD**

GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A	
Control	4 / week	4 Hr/Day								
			mean	3.45	7.73	1.36	6.60	4.84	4.79	4.24
			sd	2.16	2.47	7.41	6.02	3.29	2.43	2.48
			n	80	80	80	80	80	80	80
LowConc	2 / week	1 Hr/Day								
			mean	3.20	8.07	1.07	8.20	2.00	6.93	.40
			sd	3.95	2.34	1.79	1.70	1.65	2.05	1.55
			n	15	15	15	15	15	15	15
		4 Hr/Day								
			mean	-2.93	10.07	1.13	6.80	-.33	6.27	.
			sd	4.59	3.06	2.17	2.48	1.80	1.58	.
			n	15	15	15	15	15	15	0 <sup>a</sup>
	4 / week	1 Hr/Day								
			mean	3.53	7.93	3.33	6.40	2.87	5.33	4.27
			sd	1.88	2.22	2.53	4.14	2.67	2.64	1.83
			n	15	15	15	15	15	15	15
		4 Hr/Day								
			mean	3.67	7.87	3.60	5.27	4.07	3.87	3.73
			sd	6.30	3.72	2.41	2.43	2.05	2.59	3.03
			n	15	15	15	15	15	15	15

TABLE A-8  
(Continued)

SUMMARY OF BODY WEIGHT GAINS OF FEMALE RATS  
EXPOSURE PERIOD

GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
HighConc 2 / week 1 Hr/Day									
		mean	.40	7.53	1.87	7.73	.00	5.47	2.33
		sd	3.14	5.26	3.70	2.25	1.56	1.36	2.08
		n	15	15	15	15	15	15	3 <sup>b</sup>
4 Hr/Day									
		mean	4.27	7.07	.80	7.47	.13	6.40	1.47
		sd	2.22	2.69	2.21	1.68	1.88	2.80	2.17
		n	15	15	15	15	15	15	15
4 / week 1 Hr/Day									
		mean	3.20	5.33	3.47	5.47	5.87	2.60	4.33
		sd	1.66	7.78	2.29	4.72	1.81	2.44	1.80
		n	15	15	15	15	15	15	15
4 Hr/Day									
		mean	3.93	8.87	4.80	4.00	4.20	2.87	5.53
		sd	1.62	1.41	2.14	2.07	2.11	2.17	2.50
		n	15	15	15	15	15	15	15
PosCont 4 / week 4 Hr/Day									
		mean	2.60	6.29	3.82	4.43	3.90	3.80	3.05
		sd	4.45	6.39 <sup>c</sup>	5.05 <sup>c</sup>	3.65	3.22	2.30	4.17
		n	40	38	38	40	40	40	40

<sup>a</sup> All female rats of this exposure group were included in post-exposure assays and did not have a full three-day observation period.

<sup>b</sup> Only pulmonary function animals included. (All other female rats of this exposure group did not have a full three-day observation period because of their post-exposure assay schedule.)

<sup>c</sup> Two animals outside expected weight range excluded, affecting two weight gain periods.

ABBREVIATIONS

BWT	-	Body Weight
3DAYWK1A	-	Week 1 Period 1 (BWT gain for 3 day period in grams)
4DAYWK1B	-	Week 1 Period 2 (BWT gain for 4 day period in grams)
3DAYWK2A	-	Week 2 Period 1 (BWT gain for 3 day period in grams)
4DAYWK2B	-	Week 2 Period 2 (BWT gain for 4 day period in grams)
3DAYWK3A	-	Week 3 Period 1 (BWT gain for 3 day period in grams)
4DAYWK3B	-	Week 3 Period 2 (BWT gain for 4 day period in grams)
3DAYWK4A	-	Week 4 Period 1 (BWT gain for 3 day period in grams)

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**TABLE A-9**  
**SUMMARY OF BODY WEIGHT GAINS OF MALE RATS**  
**RECOVERY PERIOD**

GROUP	FREQUENCY	DURATION	4DAYWK48	7DAYWK5
Control	4 / week	4 Hr/Day		
		mean	9.63	17.95
		sd	3.29	5.23
		n	40	40
LowConc	2 / week	4 Hr/Day		
		mean	11.27	19.20
		sd	3.97	5.94
		n	15	15
	4 / week	1 Hr/Day		
		mean	11.00	17.00
		sd	2.93	4.58
		n	15	15
HighConc	2 / week	1 Hr/Day		
		mean	10.27	18.40
		sd	2.94	3.62
		n	15	15
	4 / week	4 Hr/Day		
		mean	10.13	21.20
		sd	3.27	4.71
		n	15	15
PosCont	4 / week	4 Hr/Day		
		mean	8.70	18.10
		sd	3.99	5.47
		n	20	20

---

**ABBREVIATIONS**

BWt - Body Weight  
4DAYWK48 - Week 4 Period 2 (BWT gain for 4 day period in grams)  
7DAYWK5 - Week 5 (BWT gain for 7 day period in grams)

**TABLE A-10**  
**SUMMARY OF BODY WEIGHT GAINS OF FEMALE RATS**  
**RECOVERY PERIOD**

GROUP	FREQUENCY	DURATION	4DAYWK4B	7DAYWK5
Control	4 / week	4 Hr/Day		
			mean	2.67
			sd	4.62
			n	39 <sup>a</sup>
LowConc	2 / week	1 Hr/Day		
			mean	5.67
			sd	1.68
			n	15
	4 / week	4 Hr/Day		
			mean	5.13
			sd	1.92
			n	15
HighConc	2 / week	4 Hr/Day		
			mean	6.07
			sd	1.67
			n	15
	4 / week	1 Hr/Day		
			mean	5.67
			sd	2.32
			n	15
PosCont	4 / week	4 Hr/Day		
			mean	3.25
			sd	3.96
			n	20

<sup>a</sup> One animal missed at one weighing, affecting two weight gain periods.

**ABBREVIATIONS**

BWT - Body Weight  
4DAYWK4B - Week 4 Period 2 (BWT gain for 4 day period in grams)  
7DAYWK5 - Week 5 (BWT gain for 7 day period in grams)

TABLE A-11

## SUMMARY OF AVERAGE DAILY FOOD CONSUMPTION OF MALE RATS

GROUP	FREQUENCY DURATION	FCWEEK1A	FCWEEK1B	FCWEEK2A	FCWEEK2B	FCWEEK3A	FCWEEK3B	FCWEEK4A	FCWEEK4B	FCWEEK5A	FCWEEK5B
Control 4 / week 4 Hr/Day											
	mean	21.66	21.31	21.03	20.76	20.41	20.61	20.87	20.82	20.41	20.97
	sd	1.59	1.84	1.72	1.23	1.47	1.44	1.32	1.39	1.72	1.29
	n	18	18	18	18	18	18	18	18	18	18
LowConc 2 / week 4 Hr/Day											
	mean	.	20.23	19.48	18.83	18.77	17.42	18.82	18.20	19.14	16.80
	sd	.	1.45	.92	1.04	.85	.78	1.54	1.33	1.11	1.37
	n	0 <sup>a</sup>	9	9	9	9	9	9	9	9	9
4 / week 1 Hr/Day											
	mean	19.14	19.07	19.53	18.23	17.43	17.03	17.40	17.69	17.81	17.38
	sd	1.53	1.38	1.26	1.00	.95	1.35	1.23	1.34	1.33	1.53
	n	7 <sup>b</sup>	9	9	9	9	9	8 <sup>c</sup>	9	9	9
HighConc 2 / week 1 Hr/Day											
	mean	22.80	21.52	19.08	18.39	17.21	17.20	18.22	.	19.66	17.88
	sd	1.84	2.10	1.42	1.31	1.08	1.16	1.54	.	1.17	1.02
	n	9	9	9	9	9	9	9	0 <sup>d</sup>	9	9
4 / week 4 Hr/Day											
	mean	20.18	18.44	18.98	19.18	18.41	18.50	18.28	18.49	17.93	18.07
	sd	1.23	1.10	.86	1.34	1.43	1.46	3.13	1.28	1.21	1.36
	n	9	9	9	9	9	9	9	9	9	9
PosCont 4 / week 4 Hr/Day											
	mean	19.33	19.78	18.49	18.18	16.92	16.65	17.05	17.13	18.24	17.71
	sd	2.21	1.40	1.71	1.74	1.64	1.46	1.51	.89	1.28	1.26
	n	12	12	12	12	12	12	12	12	12	12

<sup>a</sup> Data for this group lacked correlation with weight gain for this interval and were excluded.

<sup>b</sup> Data for two animals outside expected values excluded (see explanation for tables).

<sup>c</sup> Data for one animal outside expected values excluded (see explanation for tables).

<sup>d</sup> Data for this group unavailable because of weighing error.

## ABBREVIATIONS

FCWEEK1A	-	average daily food consumption in Week 1 Period 1
FCWEEK1B	-	average daily food consumption in Week 1 Period 2
FCWEEK2A	-	average daily food consumption in Week 2 Period 1
FCWEEK2B	-	average daily food consumption in Week 2 Period 2
FCWEEK3A	-	average daily food consumption in Week 3 Period 1
FCWEEK3B	-	average daily food consumption in Week 3 Period 2
FCWEEK4A	-	average daily food consumption in Week 4 Period 1
FCWEEK4B	-	average daily food consumption in Week 4 Period 2
FCWEEK5A	-	average daily food consumption in Week 5 Period 1
FCWEEK5B	-	average daily food consumption in Week 5 Period 2

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TABLE A-12

## SUMMARY OF AVERAGE DAILY FOOD CONSUMPTION OF FEMALE RATS

GROUP	FREQUENCY DURATION	FCWEEK1A	FCWEEK1B	FCWEEK2A	FCWEEK2B	FCWEEK3A	FCWEEK3B	FCWEEK4A	FCWEEK4B	FCWEEK5A	FCWEEK5B
Control 4 / week 4 Hr/Day											
	mean	16.84	16.44	16.00	15.84	14.74	15.57	15.34	15.40	15.37	15.46
	sd	1.09	1.22	1.32	.92	1.24	.96	1.08	1.33	1.11	1.05
	n	14 <sup>a</sup>	18	18	18	18	18	17 <sup>b</sup>	18	18	18
LowConc 2 / week 1 Hr/Day											
	mean	16.44	15.12	17.21	14.58	14.41	13.96	14.39	12.50	13.71	13.48
	sd	1.26	1.41	1.10	1.64	1.65	1.46 <sup>b</sup>	1.57 <sup>c</sup>	.85	1.17	.85
	n	9	9	9	9	9	8	7	9	9	9
4 / week 4 Hr/Day											
	mean	.	17.92	15.07	14.88	12.63	12.90	13.73	14.24	14.00	13.60
	sd	.	.96	.90	.82	.71	1.14	1.19	1.28	1.05	.80
	n	0 <sup>d</sup>	9	9	9	9	9	9	9	9	9
HighConc 2 / week 4 Hr/Day											
	mean	17.26	15.94	17.11	15.48	13.38	14.40	13.39	13.33	13.39	13.70
	sd	1.31	1.30	1.84	1.06	.91	1.09	1.04	1.56	1.14	.97
	n	9	9	9	9	9	9	9	9	9	9
4 / week 1 Hr/Day											
	mean	15.98	16.13	14.78	13.57	13.81	13.33	13.16	13.34	13.49	13.44
	sd	.44	.82	.97	1.04	.92	1.01	.80	.99	1.07	1.58
	n	9	9	9	9	9	9	9	9	9	9
PosCont 4 / week 4 Hr/Day											
	mean	17.01	15.51	14.75	14.68	13.56	13.23	13.43	13.23	12.46	13.25
	sd	2.60 <sup>b</sup>	1.42	1.33 <sup>b</sup>	1.60	1.42	.89	1.12	1.18	1.33	1.20
	n	11	12	11	12	12	12	12	12	12	12

<sup>a</sup> Data for four animals outside expected values excluded (see explanation for tables).

<sup>b</sup> Data for one animal outside expected values excluded (see explanation for tables).

<sup>c</sup> Data for two animals outside expected values excluded (see explanation for tables).

<sup>d</sup> Data for this group lacked correlation with weight gain for this interval and were excluded.

## ABBREVIATIONS

FCWEEK1A	-	average daily food consumption in Week 1 Period 1
FCWEEK1B	-	average daily food consumption in Week 1 Period 2
FCWEEK2A	-	average daily food consumption in Week 2 Period 1
FCWEEK2B	-	average daily food consumption in Week 2 Period 2
FCWEEK3A	-	average daily food consumption in Week 3 Period 1
FCWEEK3B	-	average daily food consumption in Week 3 Period 2
FCWEEK4A	-	average daily food consumption in Week 4 Period 1
FCWEEK4B	-	average daily food consumption in Week 4 Period 2
FCWEEK5A	-	average daily food consumption in Week 5 Period 1
FCWEEK5B	-	average daily food consumption in Week 5 Period 2

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**TABLE A-13**  
**SUMMARY OF LUNG/BODY WEIGHT RATIOS FOR MALE RATS**  
**POST-EXPOSURE**

GROUP	FREQUENCY	DURATION	LUNGBWT
Control	4 / week	4 Hr/Day	
			mean
			sd
			n <sup>a</sup>
LowConc	2 / week	1 Hr/Day	
			mean
			sd
			n
	4 / week	4 Hr/Day	
			mean
			sd
			n
HighConc	2 / week	4 Hr/Day	
			mean
			sd
			n
	4 / week	1 Hr/Day	
			mean
			sd
			n
PosCont	4 / week	4 Hr/Day	
			mean
			sd
			n

---

<sup>a</sup> No lung weight for one animal.

**ABBREVIATIONS**

LUNGBWT - lung to body weight ratio (x 100)

**TABLE A-14**  
**SUMMARY OF LUNG/BODY WEIGHT RATIOS FOR FEMALE RATS**  
**POST-EXPOSURE**

GROUP	FREQUENCY	DURATION	LUNGBWT
Control	4 / week	4 Hr/Day	
			mean .74
			sd .09
			n 24
LowConc	2 / week	4 Hr/Day	
			mean .75
			sd .10
			n 9
	4 / week	1 Hr/Day	
			mean .80
			sd .11
			n 9
HighConc	2 / week	1 Hr/Day	
			mean .68
			sd .11
			n 9
	4 / week	4 Hr/Day	
			mean .86
			sd .13
			n 9
PosCont	4 / week	4 Hr/Day	
			mean 1.17
			sd .18
			n 12

---

**ABBREVIATIONS**

LUNGBWT - lung to body weight ratio (x 100)

**TABLE A-15**  
**SUMMARY OF LUNG/BODY WEIGHT RATIOS FOR MALE RATS**  
**POST-RECOVERY**

GROUP	FREQUENCY	DURATION	LUNGBWT
Control	4 / week	4 Hr/Day	
			mean .73
			sd .11
			n 24
LowConc	2 / week	4 Hr/Day	
			mean .80
			sd .29
			n 9
	4 / week	1 Hr/Day	
			mean .74
			sd .14
			n 9
HighConc	2 / week	1 Hr/Day	
			mean .80
			sd .18
			n 9
	4 / week	4 Hr/Day	
			mean .83
			sd .07
			n 9
PosCont	4 / week	4 Hr/Day	
			mean 1.32
			sd .13
			n 12

---

**ABBREVIATIONS**

LUNGBWT - lung to body weight ratio (x 100)

**TABLE A-16**  
**SUMMARY OF LUNG/BODY WEIGHT RATIOS FOR FEMALE RATS**  
**POST-RECOVERY**

GROUP	FREQUENCY	DURATION	LUNGBWT
Control	4 / week	4 Hr/Day	
			mean .78
			sd .13
			n <sup>a</sup> 23
LowConc	2 / week	1 Hr/Day	
			mean .80
			sd .12
			n 9
	4 / week	4 Hr/Day	
			mean .95
			sd .16
			n 9
HighConc	2 / week	4 Hr/Day	
			mean .91
			sd .15
			n 9
	4 / week	1 Hr/Day	
			mean .91
			sd .09
			n 9
PosCont	4 / week	4 Hr/Day	
			mean 1.43
			sd .17
			n 12

---

<sup>a</sup> No lung weight for one animal.

**ABBREVIATIONS**

LUNGBWT - lung to body weight ratio (x 100)



TABLE A-17  
SUMMARY OF CLINICAL CHEMISTRY TESTS OF MALE RATS  
POST-EXPOSURE

GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP	
Control	4 / week	4 Hr/Day								
			mean	167.08	485.58	46.63	17.54	.62	154.78	6.82
			sd	56.71	30.07	5.24	2.15	.23	24.53	.47
			n	24	24	24	24	24	24	24
LowConc	2 / week	1 Hr/Day								
			mean	147.78	475.22	42.67	16.73	.54	147.58	6.43
			sd	53.60	43.79	2.40	1.82	.10	24.89	.17
			n	9	9	9	9	9	9	9
	4 / week	4 Hr/Day								
			mean	194.11	358.89	52.22 <sup>a</sup>	18.46	.64	137.93	6.83
			sd	84.41	107.39	7.74	2.27	.11	20.66	.15
			n	9	9	9	9	9	9	9
HighConc	2 / week	4 Hr/Day								
			mean	120.22	468.11	40.78	16.58	.53	147.30	6.84
			sd	31.50	45.99	2.22	1.82	.05	12.89	.19
			n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day								
			mean	188.44	456.22	48.67	17.21	.64	181.64	7.02
			sd	63.84	51.34	6.46	1.61	.09	39.65	.32
			n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day								
			mean	205.75	509.92	48.25	16.85	.58	148.92	6.83
			sd	68.84	64.96	7.59	2.71	.09	27.95	.47
			n	12	12	12	12	12	12	12

**TABLE A-17**  
(Continued)

**SUMMARY OF CLINICAL CHEMISTRY TESTS OF MALE RATS  
POST-EXPOSURE**

GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH
Control	4 / week	4 Hr/Day							
			mean	3.33	44.72	117.53	11.48	17.15	8.76
			sd	.29	4.23	28.85	.49	4.86	1.21
			n	24	24	24	24	24	24
LowConc	2 / week	1 Hr/Day							
			mean	3.09	47.86	144.79	11.09	14.88	9.53
			sd	.15	4.37	34.11	.11	3.06	1.18
			n	9	9	9	9	9	9
	4 / week	4 Hr/Day							
			mean	3.42	43.86	111.17	11.22	17.11	8.18
			sd	.16	8.49	45.50	.50	4.89	.57
			n	9	9	9	9	9	9
	2 / week	4 Hr/Day							
			mean	3.36	45.58	127.88 <sup>a</sup>	11.43	14.41	7.84
			sd	.14	2.90	25.39	.24	1.53	1.02
			n	9	9	9	9	9	9
	4 / week	1 Hr/Day							
			mean	3.47	46.50	131.02	11.49	14.43	8.04
			sd	.15	3.78	16.03	.52	2.40	.90
			n	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day							
			mean	3.35	49.93	141.46	11.54	15.88	8.37
			sd	.27	6.81	21.86	.51	5.03	.98
			n	12	12	12	12	12	12

<sup>a</sup> Corrected mean (see explanation for tables).

**ABBREVIATIONS**

CK	- creatine kinase (international units/liter serum)
ALP	- alkaline phosphatase (international units/liter serum)
ALT	- alanine aminotransferase (international units/liter serum)
BUN	- urea nitrogen (milligrams nitrogen/deciliter serum)
CREA	- Creatinine (milligrams/deciliter serum)
GLU	- glucose (milligrams/deciliter serum)
TP	- total protein (grams protein/deciliter serum)
ALBG	- albumin (grams/deciliter serum)
CHOL	- cholesterol (milligrams/deciliter serum)
TRIG	- triglycerides (milligrams/deciliter serum)
CA	- calcium (milligrams/deciliter serum)
TBA	- total bile acids (micromoles/liter serum)
PHOS	- inorganic phosphate (milligrams phosphate/deciliter serum)
SDH	- sorbitol dehydrogenase (international units/liter serum)

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**TABLE A-18**  
**SUMMARY OF CLINICAL CHEMISTRY TESTS OF FEMALE RATS**  
**POST-EXPOSURE**

GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP	
Control	4 / week	4 Hr/Day								
			mean	175.54	490.83	43.46	18.15	.56	148.50	6.56
			sd	65.80	51.22	4.18	2.06	.08	14.29	.41
			n	24	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day								
			mean	215.89	430.22	47.00	18.08	.62	153.63	6.58
			sd	96.05	44.49	13.32	1.60	.07	23.46	.29
			n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day								
			mean	135.78	457.89	38.56	18.23	.52	140.81	6.46
			sd	20.87	29.93	3.00	1.83	.05	9.16	.29
			n	9	9	9	9	9	9	9
HighConc	2 / week	1 Hr/Day								
			mean	210.11	413.78	42.33	18.22	.59	151.01	6.71
			sd	91.23	48.43	3.74	2.06	.08	22.46	.21
			n	9	9	9	9	9	9	9
	4 / week	4 Hr/Day								
			mean	152.56	452.22	41.44	15.77	.50	141.83	6.19
			sd	63.56	40.08	3.05	2.36	.08	30.01	.13
			n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day								
			mean	160.33	418.42	45.42	17.67	.58	140.50	6.26
			sd	53.48	58.38	7.59	3.57	.08	27.23	.71
			n	12	12	12	12	12	12	12

**TABLE A-18**  
(Continued)  
**SUMMARY OF CLINICAL CHEMISTRY TESTS OF FEMALE RATS**  
**POST-EXPOSURE**

GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH
Control	4 / week	4 Hr/Day							
			mean	3.21	58.29	93.58	11.35	17.54	25.63 <sup>a</sup>
			sd	.25	4.70	7.62	.42	5.40	6.82
			n	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day							
			mean	3.21	62.33	107.88 <sup>b</sup>	11.28	15.76	26.70 <sup>b</sup>
			sd	.18	6.71	18.04	.35	4.72	4.25 <sup>c</sup>
			n	9	9	9	9	9	8
	4 / week	1 Hr/Day							
			mean	3.18	62.48	106.78	11.19	16.47	19.69 <sup>b</sup>
			sd	.21	4.58	15.10	.23	1.71	4.53
			n	9	9	9	9	9	9
	2 / week	1 Hr/Day							
			mean	3.33	62.62	106.99	11.56	17.46	26.49
			sd	.07	4.41	8.40	.25	4.71	5.39
			n	9	9	9	9	9	9
	4 / week	4 Hr/Day							
			mean	2.98	62.58	107.80	10.90	15.73	20.91
			sd	.07	7.39	14.82	.17	4.58	5.30
			n	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day							
			mean	3.08	59.91	105.48	10.88	16.36	22.57 <sup>b</sup>
			sd	.40	8.89	22.33	.54	3.38	4.08
			n	12	12	12	12	12	12

<sup>a</sup> Corrected standard deviation (see explanation for tables).

<sup>b</sup> Corrected mean (see explanation for tables).

<sup>c</sup> One value more than three standard deviations from the mean excluded.

**ABBREVIATIONS**

CK	- creatine kinase (international units/liter serum)
ALP	- alkaline phosphatase (international units/liter serum)
ALT	- alanine aminotransferase (international units/liter serum)
BUN	- urea nitrogen (milligrams nitrogen/deciliter serum)
CREA	- Creatinine (milligrams/deciliter serum)
GLU	- glucose (milligrams/deciliter serum)
TP	- total protein (grams protein/deciliter serum)
ALBG	- albumin (grams/deciliter serum)
CHOL	- cholesterol (milligrams/deciliter serum)
TRIG	- triglycerides (milligrams/deciliter serum)
CA	- calcium (milligrams/deciliter serum)
TBA	- total bile acids (micromoles/liter serum)
PHOS	- inorganic phosphate (milligrams phosphate/deciliter serum)
SDH	- sorbitol dehydrogenase (international units/liter serum)

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**TABLE A-19**  
**SUMMARY OF CLINICAL CHEMISTRY TESTS OF MALE RATS**  
**POST-RECOVERY**

GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP
Control	4 / week	4 Hr/Day							
		mean	213.42	432.00	50.88	19.37	.51	152.41	6.75
		sd	79.48	44.51	7.81	2.23	.07	23.22	.23
		n	24	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day							
		mean	145.56	424.67	43.89	19.02	.52	147.72	6.72
		sd	36.66	42.88	3.18	2.27	.07	16.19	.11
		n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day							
		mean	298.44	406.00	44.00	18.53	.54	168.22	6.50
		sd	200.56	37.08	4.50	1.77	.06	25.21	.19
		n	9	9	9	9	9	9	9
HighConc	2 / week	1 Hr/Day							
		mean	161.50	419.50	44.00	19.09	.53	166.99	6.89
		sd <sup>a</sup>	49.84	46.81	3.42	.68	.04	34.73	.10
		n	8	8	8	8	8	8	8
	4 / week	4 Hr/Day							
		mean	152.44	424.56	44.44	17.12	.59	156.76	6.79
		sd	24.36	29.65	2.83	1.78	.04	33.32	.08
		n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day							
		mean	243.27	430.09	47.18	18.13	.57	137.01	6.69
		sd	98.64	25.40	3.57	2.50	.05	19.10	.16
		n <sup>a</sup>	11	11	11	11	11	11	11

**TABLE A-19**  
(Continued)

**SUMMARY OF CLINICAL CHEMISTRY TESTS OF MALE RATS  
POST-RECOVERY**

GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH
Control	4 / week	4 Hr/Day							
		mean	3.29	44.53	132.82	11.76	21.22	8.77	20.93
		sd	.15	3.38	26.49	.23	6.14	.83	5.93
		n	24	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day							
		mean	3.39	49.26	166.26	11.56	16.94	7.71	18.07
		sd	.09	5.20	24.01	.20	2.46	.50	2.76
		n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day							
		mean	3.06	45.97	133.97	11.41	20.07	9.80	21.47
		sd	.11	4.39	21.97	.42	4.19	.91	3.60
		n	9	9	9	9	9	9	9
HighConc	2 / week	1 Hr/Day							
		mean	3.31	46.38	151.85	11.89	16.44	8.55	18.05
		sd	.06	4.29	21.65	.36	1.54	.72	2.39
		n <sup>a</sup>	8	8	8	8	8	8	8
	4 / week	4 Hr/Day							
		mean	3.19	47.82	124.93	11.62	25.38	8.27	20.24
		sd	.06	2.08	18.29	.20	5.88	.75	4.32
		n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day							
		mean	3.20	43.91	135.75	11.69	19.33	8.45	22.87
		sd	.10	5.37	22.71	.20	5.13	1.51	6.94
		n <sup>a</sup>	11	11	11	11	11	11	11

<sup>a</sup> No specimen collected for one animal.

**ABBREVIATIONS**

CK	- creatine kinase (international units/liter serum)
ALP	- alkaline phosphatase (international units/liter serum)
ALT	- alanine aminotransferase (international units/liter serum)
BUN	- urea nitrogen (milligrams nitrogen/deciliter serum)
CREA	- Creatinine (milligrams/deciliter serum)
GLU	- glucose (milligrams/deciliter serum)
TP	- total protein (grams protein/deciliter serum)
ALBG	- albumin (grams/deciliter serum)
CHOL	- cholesterol (milligrams/deciliter serum)
TRIG	- triglycerides (milligrams/deciliter serum)
CA	- calcium (milligrams/deciliter serum)
TBA	- total bile acids (micromoles/liter serum)
PHOS	- inorganic phosphate (milligrams phosphate/deciliter serum)
SDH	- sorbitol dehydrogenase (international units/liter serum)

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**TABLE A-20**  
**SUMMARY OF CLINICAL CHEMISTRY TESTS OF FEMALE RATS**  
**POST-RECOVERY**

GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP
Control	4 / week	4 Hr/Day							
			mean	175.29	431.29	47.42	19.50	.52	140.88
			sd	53.71	39.54	6.51	1.34	.06	13.79
			n	24	24	24	24	24	24
LowConc	2 / week	1 Hr/Day							
			mean	182.56	403.78	41.56	17.54	.60	144.64
			sd	84.70	44.13	3.78	1.41	.06	14.89
			n	9	9	9	9	9	9
	4 / week	4 Hr/Day							
			mean	201.44	426.00	43.89	18.97	.52	150.10
			sd	89.21	17.89	2.20	2.55	.07	34.44
			n	9	9	9	9	9	9
HighConc	2 / week	4 Hr/Day							
			mean	245.89	407.11	40.89	18.42	.54	148.72
			sd	192.25	51.29	3.44	1.54	.05	28.01
			n	9	9	9	9	9	9
	4 / week	1 Hr/Day							
			mean	145.78	426.56	41.67	19.57	.54	148.19
			sd	45.63	49.06	4.85	1.34	.08	27.61
			n	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day							
			mean	219.92	426.75	41.75	18.30	.55	131.64
			sd	112.03	31.46	3.91	1.68	.06	8.33
			n	12	12	12	12	12	12

TABLE A-20  
(Continued)

SUMMARY OF CLINICAL CHEMISTRY TESTS OF FEMALE RATS  
POST-RECOVERY

GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH
Control	4 / week	4 Hr/Day							
		mean	3.18	54.71	89.75	11.27	21.64	7.47	25.50
		sd	.20	5.92	9.71	.38	5.44	1.10	5.26
		n	24	24	24	24	24	24	24
LowConc	2 / week	1 Hr/Day							
		mean	3.22	56.68	102.43	11.60	25.24	8.13	23.69
		sd	.14	4.14	13.64	.25	3.05	.79	4.53
		n	9	9	9	9	9	9	9
	4 / week	4 Hr/Day							
		mean	3.29	56.13	102.48	11.58	15.42	6.96	25.78
		sd	.18	5.47	13.39	.34	2.10	.47	4.26
		n	9	9	9	9	9	9	9
HighConc	2 / week	4 Hr/Day							
		mean	3.02	52.87	112.01	11.28	19.13	8.40	21.64
		sd	.07	4.87	17.01	.38	3.06	1.36	2.10
		n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day							
		mean	3.40	53.98	101.68	11.53	23.46	6.87	24.42
		sd	.16	5.85	10.87	.27	7.01	.93	4.60
		n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day							
		mean	3.20	50.99	100.71	11.39	17.88	7.58	24.70
		sd	.19	5.75	9.43	.23	5.14	.76	3.37
		n	12	12	12	12	12	12	12

ABBREVIATIONS

CK	- creatine kinase (international units/liter serum)
ALP	- alkaline phosphatase (international units/liter serum)
ALT	- alanine aminotransferase (international units/liter serum)
BUN	- urea nitrogen (milligrams nitrogen/deciliter serum)
CREA	- Creatinine (milligrams/deciliter serum)
GLU	- glucose (milligrams/deciliter serum)
TP	- total protein (grams protein/deciliter serum)
ALBG	- albumin (grams/deciliter serum)
CHOL	- cholesterol (milligrams/deciliter serum)
TRIG	- triglycerides (milligrams/deciliter serum)
CA	- calcium (milligrams/deciliter serum)
TBA	- total bile acids (micromoles/liter serum)
PHOS	- inorganic phosphate (milligrams phosphate/deciliter serum)
SDH	- sorbitol dehydrogenase (international units/liter serum)

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TABLE A-21

SUMMARY OF HEMATOLOGY TESTS OF MALE RATS  
POST-EXPOSURE

GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	
Control	4 / week	4 Hr/Day									
		mean	9.10	8.58	16.33	44.55	51.90	19.04	36.68	794.00	
		sd	2.15	.40	.66	2.07	.45	.42	.75	45.37	
		n	24	24	24	24	24	24	24	24	
LowConc	2 / week	1 Hr/Day	mean	9.54	8.63	16.36	44.90	52.04	18.99	36.47	779.86
			sd	1.50	.42	.49	2.02	.56	.48	.67	54.55
			n <sup>a</sup>	7	7	7	7	7	7	7	7
	4 / week	4 Hr/Day	mean	9.68	9.33	17.48	48.08	51.53	18.74	36.38	755.11
			sd	1.75	.75	1.32	4.02	.45	.39	.73	62.18
			n	9	9	9	9	9	9	9	9
HighConc	2 / week	4 Hr/Day	mean	10.10	8.79	16.66	45.77	52.06	18.94	36.39	804.44
			sd	2.56	.19	.41	1.07	.44	.42	.73	45.47
			n	9	9	9	9	9	9	9	9
	4 / week	1 Hr/Day	mean	9.09	8.83	16.61	45.46	51.51	18.82	36.54	758.00
			sd	1.49	.32	.45	1.42	.52	.47	.73	38.15
			n	9	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day	mean	11.33	8.73	16.52	45.49	52.17	18.95	36.33	787.25
			sd	1.53	.56	.91	2.61	.86	.33	.58	82.17
			n	12	12	12	12	12	12	12	12

<sup>a</sup> Two clotted samples could not be analyzed.

## ABBREVIATIONS

WBC	- white blood cell count (thousands of cells/cubic millimeter blood)
RBC	- red blood cell count (millions of cells/cubic millimeter blood)
HGB	- hemoglobin (grams/deciliter serum)
HCT	- hematocrit (percent)
MCV	- mean corpuscular volume (cubic microns)
MCH	- mean corpuscular hemoglobin (picograms)
MCHC	- mean corpuscular hemoglobin concentration (percent)
PLT	- platelet count (thousands of cells/cubic millimeter blood)

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**TABLE A-22**  
**SUMMARY OF HEMATOLOGY TESTS OF FEMALE RATS**  
**POST-EXPOSURE**

GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	
Control	4 / week	4 Hr/Day									
			mean	9.75	8.42	16.47	44.75	53.15	19.56	36.80	799.33
			sd	2.01	.36	.52	1.61	.79	.56	.78	50.42
			n	24	24	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day									
			mean	9.81	8.61	16.73	45.06	52.34	19.46	37.18	723.33
			sd	1.98	.43	.55	2.08	.43	.43	.75	41.75
			n	9	9	9	9	9	9	9	9
	4 / week	1 Hr/Day									
			mean	10.32	8.60	16.66	45.36	52.76	19.38	36.73	790.00
			sd	1.70	.34	.59	1.72	.46	.32	.53	65.06
			n	9	9	9	9	9	9	9	9
HighConc	2 / week	1 Hr/Day									
			mean	9.26	8.73	16.79	45.66	52.30	19.24	36.78	770.00
			sd	2.13	.29	.41	1.31	.42	.36	.65	74.77
			n	9	9	9	9	9	9	9	9
	4 / week	4 Hr/Day									
			mean	10.34	8.48	16.47	44.51	52.49	19.41	37.01	745.89
			sd	2.31	.21	.34	.89	.58	.26	.56	51.63
			n	9	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day									
			mean	11.94	8.06	15.57	42.30	52.62	19.37	36.84	725.67
			sd	1.96	1.62	3.01	8.25	.88	.62	1.10	79.81
			n	12	12	12	12	12	12	12	12

**ABBREVIATIONS**

WBC	- white blood cell count (thousands of cells/cubic millimeter blood)
RBC	- red blood cell count (millions of cells/cubic millimeter blood)
HGB	- hemoglobin (grams/deciliter serum)
HCT	- hematocrit (percent)
MCV	- mean corpuscular volume (cubic microns)
MCH	- mean corpuscular hemoglobin (picograms)
MCHC	- mean corpuscular hemoglobin concentration (percent)
PLT	- platelet count (thousands of cells/cubic millimeter blood)

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TABLE A-23

SUMMARY OF HEMATOLOGY TESTS OF MALE RATS  
POST-RECOVERY

GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Control	4 / week	4 Hr/Day								
			mean	10.43	8.47	15.71	42.85	50.60	18.56	781.79
			sd	1.49	.33	.35	1.69	.65	.51	34.67
			n	24	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day	mean	12.78	8.54	15.72	42.43	49.69	18.40	816.22
			sd	1.60	.21	.38	1.13	.45	.33	30.46
			n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day	mean	10.72	8.38	15.56	42.69	50.94	18.59	801.44
			sd	2.08	.35	.51	1.87	.55	.38	63.09
			n	9	9	9	9	9	9	9
HighConc	2 / week	1 Hr/Day	mean	10.84	8.58	15.83	42.89	49.96	18.46	846.75
			sd <sup>a</sup>	1.46	.29	.58	1.69	.40	.39	47.39
			n	8	8	8	8	8	8	8
	4 / week	4 Hr/Day	mean	11.87	8.57	15.63	43.67	50.98	18.29	827.33
			sd	1.96	.33	.32	1.57	.29	.44	17.47
			n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day	mean	13.42	8.81	16.15	44.55	50.57	18.34	820.27
			sd <sup>a</sup>	2.35	.27	.45	1.64	.75	.36	38.54
			n	11	11	11	11	11	11	11

<sup>a</sup> No specimen for one animal.

## ABBREVIATIONS

WBC	- white blood cell count (thousands of cells/cubic millimeter blood)
RBC	- red blood cell count (millions of cells/cubic millimeter blood)
HGB	- hemoglobin (grams/deciliter serum)
HCT	- hematocrit (percent)
MCV	- mean corpuscular volume (cubic microns)
MCH	- mean corpuscular hemoglobin (picograms)
MCHC	- mean corpuscular hemoglobin concentration (percent)
PLT	- platelet count (thousands of cells/cubic millimeter blood)

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TABLE A-24

SUMMARY OF HEMATOLOGY TESTS OF FEMALE RATS  
POST-RECOVERY

GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	
Control	4 / week	4 Hr/Day									
			mean	10.37	8.08	15.71	42.18	52.20	19.47	37.29	769.96
			sd	1.58	.42	.51	1.99	.62	.68	1.19	48.91
			n	24	24	24	24	24	24	24	24
LowConc	2 / week	1 Hr/Day									
			mean	10.03	8.35	16.03	43.64	52.29	19.22	36.76	833.00
			sd	1.46	.27	.37	1.35	.40	.57	1.00	58.11
			n	9	9	9	9	9	9	9	9
	4 / week	4 Hr/Day									
			mean	10.76	7.99	15.64	41.72	52.18	19.59	37.51	882.00
			sd	1.49	.24	.42	1.30	.47	.42	.63	47.41
			n	9	9	9	9	9	9	9	9
HighConc	2 / week	4 Hr/Day									
			mean	10.61	8.29	15.62	43.04	51.93	18.86	36.31	832.56
			sd	.88	.30	.45	1.37	.41	.53	.93	56.05
			n	9	9	9	9	9	9	9	9
	4 / week	1 Hr/Day									
			mean	10.53	7.84	15.61	40.59	51.77	19.90	38.48	848.56
			sd	1.53	.26	.41	1.29	.48	.35	.71	50.97
			n	9	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day									
			mean	12.62	8.20	15.87	42.82	52.23	19.37	37.07	834.33
			sd	2.24	.29	.45	1.65	.51	.57	1.28	59.34
			n	12	12	12	12	12	12	12	12

## ABBREVIATIONS

WBC	- white blood cell count (thousands of cells/cubic millimeter blood)
RBC	- red blood cell count (millions of cells/cubic millimeter blood)
HGB	- hemoglobin (grams/deciliter serum)
HCT	- hematocrit (percent)
MCV	- mean corpuscular volume (cubic microns)
MCH	- mean corpuscular hemoglobin (picograms)
MCHC	- mean corpuscular hemoglobin concentration (percent)
PLT	- platelet count (thousands of cells/cubic millimeter blood)

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TABLE A-25

SUMMARY OF HEMATOLOGY WBC DIFFERENTIAL COUNTS OF MALE RATS  
POST-EXPOSURE

GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT
Control	4 / week	4 Hr/Day								
			mean	9.10	.05	1.25	7.50	.29	.06	0.00
			sd	2.15	.08	.51	1.96	.17	.09	0.00
			n	24	24	24	24	24	24	24
LowConc	2 / week	1 Hr/Day								
			mean	9.54	.01	1.41	7.79	.29	.09	0.00
			sd	1.50	.04	.29	1.17	.12	.07	0.00
			n <sup>a</sup>	7	7	7	7	7	7	7
	4 / week	4 Hr/Day								
			mean	9.68	.02	1.47	7.79	.33	.09	0.00
			sd	1.75	.04	.37	1.62	.14	.08	0.00
			n	9	9	9	9	9	9	9
HighConc	2 / week	4 Hr/Day								
			mean	10.10	.02	1.59	8.10	.33	.10	0.00
			sd	2.56	.07	.52	2.46	.13	.07	0.00
			n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day								
			mean	9.09	.03	1.51	7.21	.32	.04	0.00
			sd	1.49	.05	.30	1.41	.14	.05	0.00
			n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day								
			mean	11.33	.03	2.24	8.58	.40	.11	0.00
			sd	1.53	.05	.61	1.36	.19	.09	0.00
			n	12	12	12	12	12	12	12

<sup>a</sup> Two clotted samples could not be analyzed.

## ABBREVIATIONS

WBC - white blood cell count (thousands of cells/cubic millimeter blood)  
 NRBC - nucleated red blood cells (number/100 white blood cells)  
 NEUT - neutrophils (percent leukocytes counted)  
 LYMPH - lymphocytes (percent leukocytes counted)  
 MONO - monocytes (percent leukocytes counted)  
 EOS - eosinophils (percent leukocytes counted)  
 BASO - basophils (percent leukocytes counted)  
 IM NEUT - immature neutrophils (percent leukocytes counted)

TABLE A-26

SUMMARY OF HEMATOLOGY WBC DIFFERENTIAL COUNTS OF FEMALE RATS  
POST-EXPOSURE

GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT
Control	4 / week	4 Hr/Day								
			mean	9.75	.05	1.07	8.38	.26	.05	0.00
			sd	2.01	.08	.42	1.75	.12	.06	0.00
			n	24	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day								
			mean	9.81	.03	1.38	8.06	.28	.09	0.00
			sd	1.98	.05	.49	1.64	.10	.06	0.00
			n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day								
			mean	10.32	.01	1.13	8.84	.26	.09	0.00
			sd	1.70	.03	.32	1.63	.09	.08	0.00
			n	9	9	9	9	9	9	9
HighConc	2 / week	1 Hr/Day								
			mean	9.26	.06	1.20	7.73	.26	.07	0.00
			sd	2.13	.10	.33	2.20	.09	.07	0.00
			n	9	9	9	9	9	9	9
	4 / week	4 Hr/Day								
			mean	10.34	.04	1.49	8.43	.33	.08	0.00
			sd	2.31	.07	.34	1.94	.16	.08	0.00
			n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day								
			mean	11.94	.12	2.48	8.89	.44	.11	0.00
			sd	1.96	.16	.58	1.66	.17	.07	0.00
			n	12	12	12	12	12	12	12

## ABBREVIATIONS

WBC	- white blood cell count (thousands of cells/cubic millimeter blood)
NRBC	- nucleated red blood cells (number/100 white blood cells)
NEUT	- neutrophils (percent leukocytes counted)
LYMPH	- lymphocytes (percent leukocytes counted)
MONO	- monocytes (percent leukocytes counted)
EOS	- eosinophils (percent leukocytes counted)
BASO	- basophils (percent leukocytes counted)
IM NEUT	- immature neutrophils (percent leukocytes counted)

TABLE A-27

SUMMARY OF HEMATOLOGY WBC DIFFERENTIAL COUNTS OF MALE RATS  
POST-RECOVERY

GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT
Control	4 / week	4 Hr/Day								
			mean	10.43	.07	1.54	8.55	.28	.05	0.00
			sd	1.49	.08	.36	1.33	.14	.05	0.00
			n	24	24	24	24	24	24	24
LowConc	2 / week	4 Hr/Day								
			mean	12.78	.04	2.34	9.87	.44	.11	0.00
			sd	1.60	.05	.88	1.43	.10	.09	0.00
			n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day								
			mean	10.72	.04	1.57	8.80	.31	.06	0.00
			sd	2.08	.05	.47	1.91	.09	.07	0.00
			n	9	9	9	9	9	9	9
HighConc	2 / week	1 Hr/Day								
			mean	10.84	.04	1.71	8.66	.41	.04	0.00
			sd	1.46	.07	.52	.89	.20	.05	0.00
			n <sup>a</sup>	8	8	8	8	8	8	8
	4 / week	4 Hr/Day								
			mean	11.87	.10	1.86	9.62	.29	.10	0.00
			sd	1.96	.10	.57	1.69	.12	.11	0.00
			n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day								
			mean	13.42	.20	2.81	10.12	.43	.07	0.00
			sd	2.35	.24	.46	1.90	.21	.06	0.00
			n <sup>a</sup>	11	11	11	11	11	11	11

<sup>a</sup> No specimen for one animal.

## ABBREVIATIONS

WBC - white blood cell count (thousands of cells/cubic millimeter blood)  
 NRBC - nucleated red blood cells (number/100 white blood cells)  
 NEUT - neutrophils (percent leukocytes counted)  
 LYMPH - lymphocytes (percent leukocytes counted)  
 MONO - monocytes (percent leukocytes counted)  
 EOS - eosinophils (percent leukocytes counted)  
 BASO - basophils (percent leukocytes counted)  
 IM NEUT - immature neutrophils (percent leukocytes counted)

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TABLE A-28

SUMMARY OF HEMATOLOGY WBC DIFFERENTIAL COUNTS OF FEMALE RATS  
POST-RECOVERY

GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT
Control	4 / week	4 Hr/Day								
			mean	10.37	.10	1.37	8.67	.29	.04	0.00
			sd	1.58	.13	.44	1.48	.15	.05	0.00
			n	24	24	24	24	24	24	24
LowConc	2 / week	1 Hr/Day								
			mean	10.03	.10	1.40	8.39	.17	.06	0.00
			sd	1.46	.09	.45	1.16	.07	.05	0.00
			n	9	9	9	9	9	9	9
	4 / week	4 Hr/Day								
			mean	10.76	.06	1.88	8.50	.30	.09	0.00
			sd	1.49	.07	.73	1.27	.09	.06	0.00
			n	9	9	9	9	9	9	9
HighConc	2 / week	4 Hr/Day								
			mean	10.61	.03	1.42	8.84	.27	.07	0.00
			sd	.88	.05	.41	1.00	.10	.05	0.00
			n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day								
			mean	10.53	.12	1.63	8.62	.20	.04	0.00
			sd	1.53	.14	.42	1.34	.07	.05	0.00
			n	9	9	9	9	9	9	9
PosCont	4 / week	4 Hr/Day								
			mean	12.62	.15	3.00	9.02	.44	.15	0.00
			sd	2.24	.12	.86	1.66	.18	.11	0.00
			n	12	12	12	12	12	12	12

## ABBREVIATIONS

WBC - white blood cell count (thousands of cells/cubic millimeter blood)  
 NRBC - nucleated red blood cells (number/100 white blood cells)  
 NEUT - neutrophils (percent leukocytes counted)  
 LYMPH - lymphocytes (percent leukocytes counted)  
 MONO - monocytes (percent leukocytes counted)  
 EOS - eosinophils (percent leukocytes counted)  
 BASO - basophils (percent leukocytes counted)  
 IM NEUT - immature neutrophils (percent leukocytes counted)



TABLE A-29  
SUMMARY OF CELLULAR PULMONARY LAVAGE PARAMETERS IN MALE AND FEMALE RATS  
POST EXPOSURE AND POST RECOVERY

Period	Exposure Group	Exposure Frequency	Total Viable Cells	Total Cells	Percent Viable Cells	Percent Monocytes	Percent Lymphocytes	Percent Neutrophils
Exposure	Control	4/Week <sup>a</sup>	1.492E+07 <sup>d</sup> 6.709E+06	1.568E+07 6.921E+06	95.00 2.91	97.06 3.73	1.31 1.66	1.63 3.18
	Low Dose	2/Week <sup>b</sup>	1.351E+07 2.805E+06	1.388E+07 2.655E+06	97.14 2.12	92.67 11.38	1.17 1.83	6.17 9.56
		4/Week <sup>b</sup>	1.559E+07 2.616E+06	1.608E+07 2.674E+06	96.97 3.17	85.17** 13.92	0.83 1.60	14.00** 12.65
	High Dose	2/Week <sup>b</sup>	1.704E+07 5.480E+06	1.739E+07 5.595E+06	98.03 1.60	85.83** 9.54	1.00 1.10	13.17** 9.87
		4/Week <sup>b</sup>	1.790E+07 5.078E+06	1.854E+07 5.144E+06	96.45 2.02	68.50** 11.34	1.00 1.55	30.50** 12.52
	Pos Cont	4/Week <sup>c</sup>	8.217E+07** 1.986E+07	8.719E+07** 2.133E+07	94.42 2.86	36.88** 7.49	3.00 2.83	60.13** 9.06
Recovery	Control	4/Week <sup>a</sup>	1.458E+07 2.944E+06	1.473E+07 3.026E+06	99.05 1.34	88.56 9.97	3.81 3.08	7.63 7.46
	Low Dose	2/Week <sup>b</sup>	1.804E+07 5.896E+06	1.821E+07 5.851E+06	98.86 1.40	91.33 11.06	2.83 2.79	5.83 8.45
		4/Week <sup>b</sup>	1.869E+07* 2.613E+06	1.899E+07* 2.779E+06	98.51 1.57	80.00 12.13	5.33 4.59	14.67 9.81
	High Dose	2/Week <sup>b</sup>	1.808E+07 3.877E+06	1.829E+07 3.875E+06	98.79 0.80	81.00 5.93	5.67 3.88	13.33 7.31
		4/Week <sup>b</sup>	2.050E+07** 6.394E+06	2.099E+07** 6.671E+06	97.91 2.03	80.33 10.44	5.00 5.14	14.67 10.80
	Pos Cont	4/Week <sup>c</sup>	1.389E+08** 2.157E+07	1.460E+08** 2.404E+07	95.21** 1.64	34.88** 15.08	5.88 5.38	59.25** 18.00

<sup>a</sup> Sample size = 16 rats  
<sup>b</sup> Sample size = 6 rats  
<sup>c</sup> Sample size = 8 rats  
<sup>d</sup> Mean and standard deviation of data averaged over sex and duration  
\* p<0.05 as compared to Control; One factor MANOVA  
\*\* p<0.01 as compared to Control; One factor MANOVA

**TABLE A-30**  
**SUMMARY OF ALVEOLAR MACROPHAGE PHAGOCYTOSIS**  
**AND PULMONARY LAVAGE FLUID PROTEIN DATA**  
**IN MALE AND FEMALE RATS**  
**POST EXPOSURE AND POST RECOVERY**

Period	Exposure Group	Exposure Frequency	Phagocytosis (Standardized CPM) <sup>d</sup>	Lavage Fluid Protein (ug/ml)
Exposure	Control	4/Week <sup>a</sup>	50000.00 <sup>e</sup>	63.44
			2041.90	55.61
	Low Dose	2/Week <sup>b</sup>	48699.66	70.98
			1241.95	47.68
		4/Week <sup>b</sup>	49450.44	94.18
			2559.56	78.28
	High Dose	2/Week <sup>b</sup>	49326.89	91.67
			2347.94	61.18
		4/Week <sup>b</sup>	47669.20	146.73*
			3090.98	64.23
	Pos Cont	4/Week <sup>c</sup>	47985.80	504.70**
			6701.35	139.00
Recovery	Control	4/Week <sup>a</sup>	50000.00	112.49
			4766.88	44.77
	Low Dose	2/Week <sup>b</sup>	52516.72	121.63
			6763.97	82.35
		4/Week <sup>b</sup>	47956.14	168.87
			3876.93	66.09
	High Dose	2/Week <sup>b</sup>	49600.92	208.12
			4363.51	88.82
		4/Week <sup>b</sup>	48350.86	172.53
			3097.51	84.49
	Pos Cont	4/Week <sup>c</sup>	45606.20 <sup>f</sup>	6071.60**
			4828.32	14082.00

<sup>a</sup> Sample size = 16 rats

<sup>b</sup> Sample size = 6 rats

<sup>c</sup> Sample size = 8 rats unless otherwise indicated

<sup>d</sup> Data were standardized by adding the difference between 50,000 and the mean of the filtered air control cpm value to each sample

<sup>e</sup> Mean and standard deviation of data averaged over sex and duration

<sup>f</sup> Sample size = 7 rats (1 sample deleted because of inadequate lysis)

\* P<0.05 as compared to Control; One factor MANOVA

\*\* P<0.01 as compared to Control; One factor MANOVA

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TABLE A-31  
EFFECT OF EXPOSURE ON DYNAMIC COMPLIANCE

Group	N	Dynamic Compliance (ml/cm H <sub>2</sub> O)
<u>Post-Exposure</u>		
Control	16	0.31 ± 0.16
100 mg/m <sup>3</sup>	12	0.25 ± 0.06
200 mg/m <sup>3</sup>	12	0.26 ± 0.06
<u>Post-Recovery</u>		
Control	16	0.24 ± 0.09
100 mg/m <sup>3</sup>	12	0.22 ± 0.05
200 mg/m <sup>3</sup>	11	0.23 ± 0.05

---

Data are means ± SD.

TABLE A-32

INTERACTION BETWEEN CONCENTRATION AND PERIOD (POST-EXPOSURE VERSUS POST-RECOVERY) FOR TOTAL LUNG CAPACITY AND VITAL CAPACITY

Concentration	Post-Exposure	Post-Recovery
<u>Total Lung Capacity (ml)</u>		
100 mg/m <sup>3</sup>	8.87 ± 0.87	9.16 ± 1.26
200 mg/m <sup>3</sup>	8.39 ± 0.93	9.69 ± 1.07
<u>Vital Capacity (ml)</u>		
100 mg/m <sup>3</sup>	7.65 ± 0.67	8.03 ± 1.05
200 mg/m <sup>3</sup>	7.34 ± 0.71	8.41 ± 0.83

---

Data are means ± SD.N=11 in all groups except recovery 100 mg/m<sup>3</sup> group, N=12

TABLE A-33

INTERACTION BETWEEN DURATION AND FREQUENCY FOR VITAL CAPACITY  
AND CHORD COMPLIANCE

	Duration	Frequency 2 / week	Frequency 4 / week
<u>Vital Capacity (ml)</u>			
	1 hr/day	7.67 $\pm$ 1.23	8.39 $\pm$ 1.10
	4 hr/day	8.45 $\pm$ 0.79	7.95 $\pm$ 1.31
<u>Chord Compliance</u> <u>(ml/cm H<sub>2</sub>O)</u>			
	1 hr/day	0.48 $\pm$ 0.08	0.53 $\pm$ 0.08
	4 hr/day	0.53 $\pm$ 0.05	0.49 $\pm$ 0.10

---

Data are means  $\pm$  SD.

N=12 in all groups except the 4 hr/week, 1 hr/day group, N=11.

TABLE A-34

INTERACTION BETWEEN SEX AND DURATION FOR FORCED VITAL CAPACITY  
AND FORCED EXPIRATORY VOLUME AT 100 MSEC

Duration	Male	Female
<u>Forced Vital Capacity (ml)</u>		
1 hr/day	8.66 $\pm$ 0.65	6.62 $\pm$ 0.45
4 hr/day	8.31 $\chi$ 0.68	7.32 $\chi$ 0.50
<u>Forced Expiratory Volume</u> <u>in 100 msec (ml)</u>		
1 hr/day	6.43 $\pm$ 0.65	5.28 $\pm$ 0.54
4 hr/day	5.95 $\pm$ 0.73	5.61 $\pm$ 0.37

---

Data are means  $\pm$  SD.

N=12 in all groups except Female, 1 hr/day group, N=11.

TABLE A-35

LIST OF ABBREVIATIONS ACCORDING TO SUBJECT AREAS

Inhalation Exposure

A1	-	graphite test article
C0	-	filtered-air control
C1	-	graphite @ 100 mg/m <sup>3</sup>
C2	-	graphite @ 200 mg/m <sup>3</sup>
CP	-	crystobalite @ 200 mg/m <sup>3</sup>
HighConc	-	graphite @ 200 mg/m <sup>3</sup>
LowConc	-	graphite @ 100 mg/m <sup>3</sup>
PosCont	-	crystobalite @ 200 mg/m <sup>3</sup>

Experimental Design

D1	-	1 hour/day
D2	-	4 hours/day
EXP	-	post-exposure
F1	-	two exposures/week
F2	-	four exposures/week
LAV	-	designated for pulmonary lavage
PATH	-	designated for pathology, clinical pathology and (recovery rats only) food consumption
PF	-	designated for pulmonary function tests
REC	-	post-recovery

Body Weight Gain

3DAYWK1A	-	Week 1 Period 1 (BWT gain for 3 day period in grams)
4DAYWK1B	-	Week 1 Period 2 (BWT gain for 4 day period in grams)
3DAYWK2A	-	Week 2 Period 1 (BWT gain for 3 day period in grams)
4DAYWK2B	-	Week 2 Period 2 (BWT gain for 4 day period in grams)
3DAYWK3A	-	Week 3 Period 1 (BWT gain for 3 day period in grams)
4DAYWK3B	-	Week 3 Period 2 (BWT gain for 4 day period in grams)
3DAYWK4A	-	Week 4 Period 1 (BWT gain for 3 day period in grams)
4DAYWK4B	-	Week 4 Period 2 (BWT gain for 4 day period in grams)
7DAYWK5	-	Week 5 (BWT gain for 7 day period in grams)

**TABLE A-35**  
(Continued)

**LIST OF ABBREVIATIONS ACCORDING TO SUBJECT AREAS**

**Lung Weight**

LUNGBWT - lung to body weight ratio (x 100)

**Food Consumption**

FCWEEK1A	-	average daily food consumption in Week 1 Period 1
FCWEEK1B	-	average daily food consumption in Week 1 Period 2
FCWEEK2A	-	average daily food consumption in Week 2 Period 1
FCWEEK2B	-	average daily food consumption in Week 2 Period 2
FCWEEK3A	-	average daily food consumption in Week 3 Period 1
FCWEEK3B	-	average daily food consumption in Week 3 Period 2
FCWEEK4A	-	average daily food consumption in Week 4 Period 1
FCWEEK4B	-	average daily food consumption in Week 4 Period 2
FCWEEK5A	-	average daily food consumption in Week 5 Period 1
FCWEEK5B	-	average daily food consumption in Week 5 Period 2

**Clinical Chemistry**

ALBG	-	albumin (grams/deciliter serum)
CHOL	-	cholesterol (milligrams/deciliter serum)
TRIG	-	triglycerides (milligrams/deciliter serum)
CA	-	calcium (milligrams/deciliter serum)
TBA	-	total bile acids (micromoles/liter serum)
PHOS	-	inorganic phosphate (milligrams phosphate/deciliter serum)
SDH	-	sorbitol dehydrogenase (international units/liter serum)
CK	-	creatine kinase (international units/liter serum)
ALP	-	alkaline phosphatase (international units/liter serum)
ALT	-	alanine aminotransferase (international units/liter serum)
BUN	-	urea nitrogen (milligrams nitrogen/deciliter serum)
CREA	-	Creatinine (milligrams/deciliter serum)
GLU	-	glucose (milligrams/deciliter serum)
TP	-	total protein (grams protein/deciliter serum)



**TABLE A-35**  
(Continued)

**LIST OF ABBREVIATIONS ACCORDING TO SUBJECT AREAS**

**Hematology**

WBC	- white blood cell count (thousands of cells/cubic millimeter blood)
RBC	- red blood cell count (millions of cells/cubic millimeter blood)
HGB	- hemoglobin (grams/deciliter serum)
HCT	- hematocrit (percent)
MCV	- mean corpuscular volume (cubic microns)
MCH	- mean corpuscular hemoglobin (picograms)
MCHC	- mean corpuscular hemoglobin concentration (percent)
PLT	- platelet count (thousands of cells/cubic millimeter blood)

**Hematology Differential Count**

WBC	- white blood cell count (thousands of cells/cubic millimeter blood)
NRBC	- nucleated red blood cells (number/100 white blood cells)
NEUT	- neutrophils (percent leukocytes counted)
LYMPH	- lymphocytes (percent leukocytes counted)
MONO	- monocytes (percent leukocytes counted)
EOS	- eosinophils (percent leukocytes counted)
BASO	- basophils (percent leukocytes counted)
IM NEUT	- immature neutrophils (percent leukocytes counted)

**Pulmonary Lavage Parameters**

AM	- alveolar macrophage
CRBC	- <sup>51</sup> Chromium-labelled chicken red blood cells
PERVCELL	- percent viable cells (proportion viable)
PHAGO	- phagocytosis (counts per minute)
PROTEIN	- lavage fluid protein (micrograms/milliliter)
STDPHAGO	- standardized phagocytosis (counts per minute)
TOTVCELL	- total viable cells
TOTCELL	- total cells

TABLE A-35  
(Continued)

LIST OF ABBREVIATIONS ACCORDING TO SUBJECT AREAS

Pulmonary Function

Cchord	- compliance (tangent) at 0 to 10 cm H <sub>2</sub> O (ml/cm H <sub>2</sub> O)
Cdyn	- dynamic compliance (ml/cm H <sub>2</sub> O)
Cpk	- peak compliance (ml/cm H <sub>2</sub> O)
DL <sub>CO</sub>	- diffusion capacity for carbon monoxide (ml/min x torr)
FEF75	- forced expiratory flow at 75% of remaining FVC (ml/sec)
FEF50	- forced expiratory flow at 50% of remaining FVC (ml/sec)
FEF25	- forced expiratory flow at 25% of remaining FVC (ml/sec)
FEV50	- forced expiratory volume at 50 msec of expiration (ml @ 50 msec)
FEV100	- forced expiratory volume at 100 msec of expiration (ml @ 100 msec)
FEV200	- forced expiratory volume at 200 msec of expiration (ml @ 200 msec)
FEV400	- forced expiratory volume at 400 msec of expiration (ml @ 400 msec)
FOB	- frequency of breathing (breaths/min)
FVC	- forced vital capacity (ml)
MMEXF	- mean mid-expiratory flow (ml/sec)
Pes	- esophageal pressure (cm H <sub>2</sub> O)
PEXF	- peak expiratory flow (ml/sec)
Rl	- resistance (cm H <sub>2</sub> O/ml x sec)
RV	- residual volume (ml)
Te	- expiratory time (sec)
Ti	- inspiratory time (sec)
TLC	- total lung capacity (ml)
VC	- vital capacity (ml)
VCpv	- vital capacity (pressure-volume derived) (ml)
VE	- minute volume (ml/min)
Vemax	- maximum flow during tidal expiration (ml/sec)
Vimax	- maximum flow during tidal inspiration (ml/sec)
VT	- tidal volume (ml)
VPEXF	- volume at PEXF (ml)

PART ONE

APPENDIX B: PATHOLOGY REPORT

IIT RESEARCH INSTITUTE

PATHOLOGY REPORT  
FOR  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

PREPARED  
BY  
PATHOLOGY ASSOCIATES, INC.  
10 WEST 35TH STREET  
CHICAGO, IL 60616

FOR  
IIT RESEARCH INSTITUTE  
10 WEST 35TH STREET  
CHICAGO, ILLINOIS 60616-3799

MARCH 11, 1991

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Pathology Report  
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SECTION I  
PATHOLOGY NARRATIVE

## PATHOLOGY REPORT

### FOUR WEEK INHALATION TOXICITY STUDY OF A SOLID PARTICULATE AEROSOL IN F344/N RATS

#### INTRODUCTION

This report by Pathology Associates, Inc. (PAI) to IIT Research Institute, (IITRI), 10 West 35th Street, Chicago, Illinois, 60616-3799, presents the results of pathology support for IITRI Project Number L06234, Study Number 1.

#### EXPERIMENTAL DESIGN AND METHODS

In the core study seventy-two male and seventy-two female F344/N rats were exposed to the test article (graphite), filtered air (negative control) or positive control test material (crystalline silica, 400 mesh) by whole body inhalation. Ten treatment groups were used to study the effects of three primary (concentration, duration, frequency) and two secondary (sex, recovery) factors on response to the test article. Treatment groups are given in Attachment 1, Summary of Experimental Design. At the conclusion of the 28 day exposure period, all core study rats were killed via asphyxiation with carbon dioxide and subjected to a complete necropsy.

In the recovery study, number of animals per group and exposure conditions were identical to the core study but these rats were allowed a 14 day recovery period following the last exposure before being killed and necropsied.

All protocol required tissues (see Attachment 2) were preserved in 10% neutral buffered formalin. After thorough fixation, all protocol required tissues from animals in the core and recovery studies were processed through paraffin, sectioned at approximately 5  $\mu$ m and stained with hematoxylin and eosin (H&E). As per protocol, tissues from animals in Groups I, IX and X in the core and recovery studies were examined microscopically. Based on evaluation of these tissues, no test article related lesions were identified. Thus, according to protocol instructions, tissues from animals in Groups II-VIII in the core and recovery studies were not examined. Bronchial lymph nodes were taken as pulmonary lymph



nodes and mediastinal lymph nodes were collected and examined only if they contained a gross lesion.

Microscopic findings for all groups examined are summarized in the Project Summary Tables (Section II). The mean group severity scores, determined by dividing the sum of all severity scores for a finding by the number of tissues examined, are found in the Severity Summary Tables (Section III). Microscopic diagnoses for protocol required tissues for individual animals are presented in the Tabulated Animal Data Table (Section IV). Microscopic diagnoses are correlated with gross lesions, when possible, in the Correlation of Gross and Microscopic (Micro) Findings Table (Section V). The codes used as entries in these tables are explained in the Reports Code Table (Attachment 3) and abbreviations used in any of the tables are explained in the Abbreviation List (Attachment 4).

## RESULTS

### Gross Lesions

Observations recorded on Individual Animal Necropsy Records as gross lesions were mostly color changes in lungs and lymph nodes (pulmonary and mediastinal lymph nodes). In the core and recovery study animals exposed to the test article, these changes were brown, gray or black discoloration. There were no histopathologic lesions related to the presence of test article in these organs. For this reason, the presence of these grossly observed color changes was interpreted as evidence of exposure to the test article rather than as a gross lesion. Enlarged pulmonary and mediastinal lymph nodes in animals which received the positive control, crystalline silica, were considered related to the granulomas seen microscopically. Other gross lesions were regarded as incidental findings and warrant no further discussion.

### Diagnostic Terms

Morphologic features of terms which follow are presented to aid in interpreting data in the tables. The terms listed here were not necessarily associated with the test article.

#### Nose

Hyperplasia of goblet cells refers to an increase in number of mucus producing goblet cells in the respiratory epithelium.

#### Lymph Nodes

Hyperplasia of lymph nodes was characterized by enlargement due to increased numbers of lymphocytes and plasma cells. Germinal centers were increased in size and number. Granulomas were foci of large activated macrophages (histiocytes) which contained abundant pale cytoplasm and a single, usually reniform nucleus.

These foci were sometimes coalescent. Multinucleated giant cells were not seen.

#### Lung

Inflammation consisted of mononuclear cell (lymphocyte, macrophage, plasma cell) and neutrophil infiltrates associated primarily with terminal and respiratory bronchioles. Minimal to mild proliferation of epithelial cells was seen in most foci but syncytial cells were not noted. Similar infiltrates of a lesser severity were seen around small blood vessels. Granulomatous inflammation within peribronchial or peribronchiolar lymphoid tissues was restricted to group X and group X-recovery animals and was similar to that seen in lymph nodes.

#### Heart

Subacute inflammation consisted of foci, often one per section, of macrophages, lymphocytes and plasma cells within the myocardium.

#### Liver

Inflammation in the liver consisted of foci of lymphocytes and plasma cells within the parenchyma.

#### Pigment

Pigment was a granular black material seen within macrophages in alveoli, interstitium and lymphoid tissue of the lung or in mediastinal or bronchial lymph nodes. It occurred only in groups receiving test article and is consistent with the test article.

The remainder of the diagnoses used in this study were considered to be self explanatory, and were not discussed in this section.

#### Histopathology

Hyperplasia of goblet cells in respiratory epithelium of nasal turbinates occurred in 5 of 12 males and in 5 of 12 females in the positive control group with a mean group severity score of 0.42 in both sexes. As this lesion did not occur in either air control or test article treated groups, it was not considered test article related.

Inflammation in lungs occurred in air control, test article and positive control groups in both core and recovery studies. Incidence and severity of this lesion were most severe in positive control animals as compared to air control and test article groups in core and recovery studies. Also, in all three groups (air control, test article and positive control), both incidence and severity were greater in recovery than in core study animals. These observations, as well as the histologic character and distribution, suggest this lesion to be a response to an unknown infectious or toxic agent. Similar lesions have been seen in other

studies in this and other laboratories but no causative agent has been proven. This lesion is not considered test article related.

Pigment was seen only in animals exposed to the test article. It occurred within alveolar macrophages or within macrophages in the interstitium and lymphoid tissue of the lung. It appeared to be inert in these locations. Pigment was also seen in pulmonary lymph nodes in all core and recovery animals exposed to the test article in which these lymph nodes were available for examination. These observations are consistent with normal clearance of inert particulate materials from the lung. Alveolar macrophages phagocytize the particles and either move up the airways with mucus to be expectorated or move into lymphatics of the interstitium and then into medullary sinuses of the draining lymph nodes. The presence of pigment in these locations is interpreted as evidence of exposure to the test article but not as a test article related lesion.

Hyperplasia of pulmonary lymph nodes occurred in only 3 of 9 animals in the core study test article group (Group IX). It was scored as minimal in animals 184 and 188 which did not have concurrent inflammation in the lung and as mild in animal 191 which did have mild inflammation in the lung. In Group IX recovery study animals, hyperplasia of pulmonary lymph node and inflammation of lung occurred in 8 of 8 and 9 of 9 animals, respectively. Hyperplasia of the pulmonary lymph nodes was thus interpreted as related to inflammation in the lung rather than related to the test article.

Granulomatous inflammation occurred in lung, intrapulmonary lymphoid tissue and pulmonary and mediastinal lymph nodes. As only core and recovery animals receiving the positive control, crystalline silica, had this lesion, it was considered to not be related to the test article.

Mediastinal lymph nodes receive lymphatics from the lungs. Changes in these nodes, as in the pulmonary lymph nodes, reflect processes in the lung. Thus, as in pulmonary lymph nodes, hyperplasia in mediastinal lymph nodes is interpreted as related to inflammation in the lung rather than related to the test article. Granulomas in mediastinal lymph nodes of positive control animals represent a response to the crystalline silica.

#### CONCLUSIONS

The presence of gray or black coloration of lung, pulmonary or mediastinal lymph nodes noted at necropsy are consistent with exposure to the test article. No microscopic lesions related to the test article were seen in core or recovery animals exposed to the most severe conditions of concentration, duration or frequency. Animals exposed to the positive control, crystalline silica, developed granulomatous inflammation in lungs and in pulmonary and mediastinal lymph nodes. Inflammation in lungs of

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air control, test article and positive control exposed animals suggested a response to an infectious or toxic agent, but is not related to the test article.

Michael J. Tomlinson, DVM, PhD  
Diplomate ACVP

Date

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ATTACHMENT 1  
SUMMARY OF EXPERIMENTAL DESIGN

SUMMARY OF EXPERIMENTAL DESIGN

Test Group	Test Article Exposure			Number of Animals			
	Concentration	Duration	Frequency	Core Study		Recovery Study	
				Male	Female	Male	Female
I	0	D2	F2	24	24	24	24
II	C1	D1	F1	9	0	0	9
III	C2	D1	F1	0	9	9	0
IV	C1	D1	F2	0	9	9	0
V	C2	D1	F2	9	0	0	9
VI	C1	D2	F1	0	9	9	0
VII	C2	D2	F1	9	0	0	9
VIII	C1	D2	F2	9	0	0	9
IX	C2	D2	F2	0	9	9	0
X	P	D2	F2	12	12	12	12

Concentration: 0 = None, C1 = 100 mg/m<sup>3</sup>, C2 = 200 mg/m<sup>3</sup>,  
P = Positive Control

Duration: D1 = 1 hr/day, D2 = 4 hr/day

Frequency: F1 = 2 days/week, F2 = 4 days/week

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ATTACHMENT 2  
PROTOCOL-REQUIRED TISSUES

PROTOCOL REQUIRED TISSUES

- \*Nasal Turbinates (3 sections)
- \*Larynx
- \*Trachea (cross and longitudinal sections)
- \*Lung (at least 3 lobes sectioned along main bronchus)
- \*Pulmonary Lymph Nodes
- Heart
- Liver
- Spleen
- Kidneys
- Urinary Bladder
- Stomach
- Adrenal Glands

\* Only these tissues required from groups II-VIII.



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ATTACHMENT 3  
REPORTS CODE TABLE

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Reports Code Table

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N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
P	Paired organ missing
U	Tissues unsuitable/unavailable for evaluation
S	Tissues not applicable to sex
*	Tissues not required by protocol

---

1	minimal
2	mild
3	moderate
4	marked
)	focal
]	locally extensive
>	multifocal
P	Present
B	Neoplasm, Benign
M	Neoplasm, Malignant without Metastasis
C	Neoplasm, Malignant with Metastasis
X	Metastatic Site (+)
-	No data entered

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ATTACHMENT 4  
ABBREVIATION LIST

HISTOPATHOLOGY TABLES

ABBREVIATION LIST

LN - LYMPH NODE

SECTION II  
PROJECT SUMMARY TABLE

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Project Summary Table**

SUMMARY: Incidence of NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: L06234  
 DAYS : 25-39

FATES: SCHEDULED SACRIFICE  
 SEX: MALE

PAGE 1

GROUP:	I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:	24	24	0	9	12	12

		#	%	#	%	#	%	#	%	#	%	#	%
NOSE	# Ex	24		24		0		9		12		12	
Hyperplasia,goblet cell		0	(0)	0	(0)	0		0	(0)	5	(42)	0	(0)
Hemorrhage		0	(0)	0	(0)	0		0	(0)	0	(0)	2	(17)
LARYNX	# Ex	24		23		0		9		12		12	
TRACHEA	# Ex	24		24		0		9		12		12	
PULMONARY LN	# Ex	21		22		0		8		10		6	
Hyperplasia		0	(0)	2	(9)	0		8	(100)	0	(0)	0	(0)
Granuloma		0	(0)	0	(0)	0		0	(0)	6	(60)	6	(100)
Pigment		0	(0)	0	(0)	0		8	(100)	0	(0)	0	(0)
Congestion		1	(5)	0	(0)	0		0	(0)	0	(0)	0	(0)
LUNGS	# Ex	24		24		0		9		12		12	
Hemorrhage		0	(0)	1	(4)	0		0	(0)	0	(0)	0	(0)
Inflammation		5	(21)	18	(75)	0		9	(100)	10	(83)	12	(100)
Inflammation,granulomatous		0	(0)	0	(0)	0		0	(0)	12	(100)	12	(100)
Pigment		0	(0)	0	(0)	0		9	(100)	0	(0)	0	(0)
Lymphoid tissue,granuloma		0	(0)	0	(0)	0		0	(0)	6	(50)	12	(100)
HEART	# Ex	24		24		0		9		12		12	
Inflammation		0	(0)	0	(0)	0		1	(11)	0	(0)	0	(0)
Inflammation,subacute		2	(8)	2	(8)	0		0	(0)	0	(0)	0	(0)
URINARY BLADDER	# Ex	24		24		0		9		12		12	
Calculus		4	(17)	0	(0)	0		1	(11)	1	(8)	1	(8)
STOMACH	# Ex	23		24		0		9		12		12	
LIVER	# Ex	24		24		0		9		12		12	
Hepatodiaphragmatic nodule		0	(0)	1	(4)	0		0	(0)	0	(0)	0	(0)
Inflammation		1	(4)	0	(0)	0		0	(0)	1	(8)	0	(0)

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Project Summary Table**

SUMMARY: Incidence of NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: L06234  
 DAYS : 25-39

FATES: SCHEDULED SACRIFICE  
 SEX: MALE

PAGE 2

GROUP:	I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:	24	24	0	9	12	12

	#	%	#	%	#	%	#	%	#	%	#	%
	Ex											
SPLEEN	24		24		0		9		12		12	
KIDNEYS	24		24		0		9		12		12	
Nephrosis	0	(0)	0	(0)	0		0	(0)	0	(0)	1	(8)
ADRENALS	24		24		0		9		12		12	
MEDIASTINAL LN	1		3		0				3		9	
Hyperplasia	0	(0)	1	(33)	0		3	(75)	0	(0)	0	(0)
Granuloma	0	(0)	0	(0)	3		0	(0)	3	(100)	9	(100)
Congestion	0	(0)	1	(33)	0		0	(0)	0	(0)	0	(0)
Pigment	0	(0)	0	(0)	0		4	(100)	0	(0)	0	(0)

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
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**Project Summary Table**

SUMMARY: Incidence of NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: L06234  
 DAYS : 25-39

FATES: SCHEDULED SACRIFICE  
 SEX: MALE

PAGE 3

GROUP:	I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:	24	24	0	9	12	12

OTHER TISSUES AND LESIONS:

	#	%	#	%	#	%	#	%	#	%	#	%
THYMUS: Normal	0	(0)	3	(13)	0	(0)	0	(0)	1	(8)	0	(0)
THYMUS: Congestion	0	(0)	1	(4)	0	(0)	0	(0)	0	(0)	0	(0)



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Project Summary Table**

SUMMARY: Incidence of NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: L06234		FATES: SCHEDULED SACRIFICE		PAGE 4			
DAYS : 25-39		SEX: FEMALE					
GROUP:		I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:		24	24	9	0	12	12
NOSE	# Ex	# %	# %	# %	# %	# %	# %
Hyperplasia,goblet cell		0 (0)	0 (0)	0 (0)	0	5 (42)	0 (0)
Hemorrhage		0 (0)	0 (0)	0 (0)	0	0 (0)	1 (8)
LARYNX	# Ex	24	24	9	0	12	12
TRACHEA	# Ex	24	24	9	0	12	12
PULMONARY LN	# Ex	20	22	9	0	12	7
Hyperplasia		0 (0)	7 (32)	3 (33)	0	1 (8)	0 (0)
Granuloma		0 (0)	0 (0)	0 (0)	0	6 (50)	7 (100)
Pigment		0 (0)	0 (0)	9 (100)	0	0 (0)	0 (0)
LUNGS	# Ex	24	24	9	0	12	12
Hemorrhage		1 (4)	0 (0)	0 (0)	0	0 (0)	0 (0)
Inflammation		5 (21)	13 (54)	4 (44)	0	9 (75)	12 (100)
Inflammation,granulomatous		0 (0)	0 (0)	0 (0)	0	12 (100)	12 (100)
Pigment		0 (0)	0 (0)	9 (100)	0	0 (0)	0 (0)
Lymphoid tissue,granuloma		0 (0)	0 (0)	0 (0)	0	2 (17)	11 (92)
HEART	# Ex	24	24	9	0	12	12
Inflammation,subacute		2 (8)	0 (0)	0 (0)	0	0 (0)	0 (0)
URINARY BLADDER	# Ex	24	24	9	0	12	12
STOMACH	# Ex	24	24	9	0	12	12
LIVER	# Ex	24	24	9	0	12	12
Hepatodlaphragmatic nodule		1 (4)	0 (0)	0 (0)	0	0 (0)	1 (8)
SPLEEN	# Ex	24	24	9	0	12	12

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Project Summary Table**

SUMMARY: Incidence of NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: L06234  
 DAYS : 25-39

FATES: SCHEDULED SACRIFICE  
 SEX: FEMALE

PAGE 5

GROUP:	I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:	24	24	9	0	12	12

	#	%	#	%	#	%	#	%	#	%	#	%	
	#	Ex	24		24		9		0		12		12
KIDNEYS	#	Ex	24		24		9		0		12		12
ADRENALS	#	Ex	24		24		9		0		12		12
MEDIASTINAL LN	#	Ex	0		2		1		0		1		11
Hyperplasia			0		1 (50)		1 (100)		0		1 (100)		0 (0)
Granuloma			0		0 (0)		0 (0)		0		0 (0)		11 (100)
Pigment			0		0 (0)		1 (100)		0		0 (0)		0 (0)

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Project Summary Table**

SUMMARY: Incidence of NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: L06234  
DAYS : 25-39

FATES: SCHEDULED SACRIFICE  
SEX: FEMALE

PAGE 6

GROUP:	I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:	24	24	9	0	12	12

OTHER TISSUES AND LESIONS:	#	%	#	%	#	%	#	%	#	%	#	%
THYMUS: Hemorrhage	0	(0)	0	(0)	1	(11)	0	(0)	0	(0)	0	(0)
THYMUS: Normal	1	(4)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
OVARY: Cyst	0	(0)	1	(4)	0	(0)	0	(0)	0	(0)	0	(0)



SECTION III  
SEVERITY SUMMARY TABLE

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Severity Summary Table

PAGE 1

PROJECT ID. NO: L06234  
 DAYS: 25-39

FATES: SCHEDULED SACRIFICE  
 SEX: MALE

GROUP:

NUMBER OF ANIMALS:

I	I-R	IX	IX-R	X	X-R
24	24	0	9	12	12

		#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
NOSE	# Ex	24		24		0		9		12	
Hyperplasia,goblet cell		0		0		0		0	0.42	0	
Hemorrhage		0		0		0		0		2	0.33
LARYNX	# Ex	24		23		0		9		12	
TRACHEA	# Ex	24		24		0		9		12	
PULMONARY LN	# Ex	21		22		0		8		10	
Hyperplasia		0		2	0.18	0		8	2.38	0	
Granuloma		0		0		0		0		6	1.70
Pigment		0		0		0		8	3.00	0	
Congestion		1	0.10	0		0		0		0	
LUNGS	# Ex	24		24		0		9		12	
Hemorrhage		0		1	0.04	0		0		0	
Inflammation		5	0.25	18	1.83	0		9	2.11	10	1.25
Inflammation,granulomatous		0		0		0		0		12	2.42
Pigment		0		0		0		9	3.00	0	
Lymphoid tissue,granuloma		0		0		0		0		6	1.00
HEART	# Ex	24		24		0		9		12	
Inflammation		0		0		0		1	0.11	0	
Inflammation,subacute		2	0.08	2	0.08	0		0		0	
URINARY BLADDER	# Ex	24		24		0		9		12	
Calculus		4	0.17	0		0		1	0.11	1	0.08
STOMACH	# Ex	23		24		0		9		12	
LIVER	# Ex	24		24		0		9		12	
Hepatodiaphragmatic nodule		0		1	0.04	0		0		0	

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Severity Summary Table

PAGE 2

PROJECT ID. NO: L06234  
DAYS: 25-39

FATES: SCHEDULED SACRIFICE  
SEX: MALE

GROUP:	I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:	24	24	0	9	12	12

	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
Inflammation	1	0.04	0		0		0		1	0.08	0	
SPLEEN	# Ex	24	24		0		9		12		12	
KIDNEYS	# Ex	24	24		0		9		12		12	
Nephrosis		0	0		0		0		0		1	0.33
ADRENALS	# Ex	24	24		0		9		12		12	
MEDIASTINAL LN	# Ex	1	3		0		4		3		9	
Hyperplasia		0	1	0.67	0		3	1.50	0		0	
Granuloma		0	0		0		0		3	3.33	9	3.89
Congestion		0	1	0.67	0		0		0		0	
Pigment		0	0		0		4	2.25	0		0	

\* Severity calculated by the number of tissues examined.

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Severity Summary Table

PAGE 3

PROJECT ID. NO: L06234  
DAYS: 25-39

FATES: SCHEDULED SACRIFICE  
SEX: FEMALE

GROUP:

NUMBER OF ANIMALS:

I	I-R	IX	IX-R	X	X-R
24	24	9	0	12	12

	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	
NOSE	# Ex	24		24		9		0		12	
Hyperplasia, goblet cell		0		0		0		0		5	0.42
Hemorrhage		0		0		0		0		0	
										1	0.17
LARYNX	# Ex	24		24		9		0		12	
TRACHEA	# Ex	24		24		9		0		12	
PULMONARY LN	# Ex	20		22		9		0		12	
Hyperplasia		0		7	0.68	3	0.44	0		1	0.25
Granuloma		0		0		0		0		6	1.17
Pigment		0		0		9	2.00	0		0	
										7	3.71
LUNGS	# Ex	24		24		9		0		12	
Hemorrhage		1	0.04	0		0		0		0	
Inflammation		5	0.29	13	1.17	4	0.78	0		9	1.17
Inflammation, granulomatous		0		0		0		0		12	2.67
Pigment		0		0		9	2.67	0		0	
Lymphoid tissue, granuloma		0		0		0		0		2	0.33
										11	2.67
HEART	# Ex	24		24		9		0		12	
Inflammation, subacute		2	0.08	0		0		0		0	
URINARY BLADDER	# Ex	24		24		9		0		12	
STOMACH	# Ex	24		24		9		0		12	
LIVER	# Ex	24		24		9		0		12	
Hepatodiaphragmatic nodule		1	0.04	0		0		0		0	
										1	0.08
SPLEEN	# Ex	24		24		9		0		12	



PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Severity Summary Table

PAGE 4

PROJECT ID. NO: L06234  
 DAYS: 25-39

FATES: SCHEDULED SACRIFICE  
 SEX: FEMALE

GROUP:	I	I-R	IX	IX-R	X	X-R
NUMBER OF ANIMALS:	24	24	9	0	12	12

	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
	#	Ex			#	Ex			#	Ex		
KIDNEYS		24		24		9		0		12		12
ADRENALS		24		24		9		0		12		12
MEDIASTINAL LN		0		2		1		0		1		11
Hyperplasia		0		1 1.00		1 2.00		0		1 3.00		0
Granuloma		0		0		0		0		0		11 3.91
Pigment		0		0		1 3.00		0		0		0

\* Severity calculated by the number of tissues examined.



SECTION IV  
TABULATED ANIMAL DATA

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PAGE 1

PROJECT ID: L06234      GROUP: I      SEX: MALE  
 DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	003	004	005	006	007	008	035	036	037	038
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN	N	N	N	N	N	N	N	N	N	N
LUNGS Inflammation	-	-	-	-	-	-	-	-	-	1
HEART Inflammation, subacute	-	-	-	-	-	1	-	1	-	-
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
KIDNEYS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Tabulated Animal Data

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PROJECT ID: L06234      GROUP: I      SEX: MALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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PAGE 2

ANIMAL ID:	003	004	005	006	007	008	035	036	037	038
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*	*	*	*	*	*	*

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 3

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: I      SEX: MALE  
 FATES: SCHEDULED SACRIFICE

ANIMAL ID:	039	040	067	068	069	070	071	072	115	116
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN	N	N	N	N	N	N	N	U	U	N
LUNGS	N	N		N	N		N	N	N	N
Inflammation	-	-	1	-	-	2	-	-	-	-
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N		N	N	N	N	N	
Calculus	-	-	-	1	-	-	-	-	-	1
STOMACH	N	U	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
KIDNEYS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 4

PROJECT ID: L06234  
DAYS: 25-39

GROUP: I  
SEX: MALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	039	040	067	068	069	070	071	072	115	116
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*	*	N	*	*	*	*

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID. L06234  
DAYS: 25-39

GROUP: I  
FATES: SCHEDULED SACRIFICE

SEX: MALE

ANIMAL ID:	117	118	119	120
NOSE	N	N	N	N
LARYNX	N	N	N	N
TRACHEA	N	N	N	N
PULMONARY LN Congestion	2	U -	N -	N -
LUNGS Inflammation	N -	1	N -	1
HEART	N	N	N	N
URINARY BLADDER Calculus	1	1	N -	N -
STOMACH	N	N	N	N
LIVER Inflammation	N -	1	N -	N -
SPLEEN	N	N	N	N



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 6

PROJECT ID: L06234  
DAYS: 25-39

GROUP: I  
FATES: SCHEDULED SACRIFICE

SEX: MALE

ANIMAL ID:	117	118	119	120
KIDNEYS	N	N	N	N
ADRENALS	N	N	N	N
MEDIASTINAL LN	*	*	*	*

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 7

PROJECT ID: L06234      GROUP: I-R      SEX: MALE  
 DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	011	012	013	014	015	016	043	044	045	046
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN Hyperplasia	N -	 2	N -	N -	N -	N -	N -	N -	N -	N -
LUNGS								N		
Hemorrhage	-	-	1	-	-	-	-	-	-	-
Inflammation	3	2	-	1	2	4	3	-	2	1
HEART	N	N	N		N	N	N	N	N	N
Inflammation, subacute	-	-	-	1	-	-	-	-	-	-
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PROJECT ID: L06234      GROUP: I-R      SEX: MALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

PAGE 8

ANIMAL ID:	011	012	013	014	015	016	043	044	045	046
KIDNEYS	N	N	N	N	N	N	N	N	N	N
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*	*	*	*	*	*	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R      SEX: MALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	011	012	013	014	015	016	043	044	045	046
OTHER TISSUES AND LESIONS:										
THYMUS: Normal	P	-	-	-	-	-	-	P	-	P

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE  
 DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	047	048	075	076	077	078	079	080	123	124
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	U	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN	N	N	N	N	N	N	N	N	N	N
LUNGS				N				N	N	
Inflammation	3	3	1	-	3	3	2	-	-	3
HEART	N	N	N	N	N	N	N	N		N
Inflammation, subacute	-	-	-	-	-	-	-	-	1	-
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
KIDNEYS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R      SEX: MALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	047	048	075	076	077	078	079	080	123	124
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*	*	*	*		*	*
Congestion	-	-	-	-	-	-	-	2	-	-

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Tabulated Animal Data

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

---

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ANIMAL ID:	047	048	075	076	077	078	079	080	123	124
OTHER TISSUES AND LESIONS:										
THYMUS: Congestion	-	-	-	-	-	-	-	-	2	-

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

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PROJECT ID: L06234  
 DAYS: 25-39

GROUP: I-R      SEX: MALE  
 FATES: SCHEDULED SACRIFICE

ANIMAL ID:	125	126	127	128
NOSE	N	N	N	N
LARYNX	N	N	N	N
TRACHEA	N	N	N	N
PULMONARY LN Hyperplasia	U -	2	U -	N -
LUNGS Inflammation	N -	4	1	3
HEART	N	N	N	N
URINARY BLADDER	N	N	N	N
STOMACH	N	N	N	N
LIVER Hepatodiaphragmatic nodule	N -	1	N -	N -
SPLEEN	N	N	N	N
KIDNEYS	N	N	N	N



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R      SEX: MALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	125	126	127	128
ADRENALS	N	N	N	N
MEDIASTINAL LN	*		*	*
Hyperplasia	-	2	-	-

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R      SEX: MALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	125	126	127	128
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OTHER TISSUES AND LESIONS:

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PAGE 16

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: IX-R      SEX: MALE  
 FATES: SCHEDULED SACRIFICE

ANIMAL ID:	164	165	166	167	168	169	170	171	172
NOSE	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N
PULMONARY LN								U	
Hyperplasia	2	3	2	2	3	2	3	-	2
Pigment	3	3	3	3	3	3	3	-	3
LUNGS									
Inflammation	4	2	3	1	1	1	2	3	2
Pigment	3	3	3	3	3	3	3	3	3
HEART	N	N		N	N	N	N	N	N
Inflammation	-	-	1	-	-	-	-	-	-
URINARY BLADDER	N	N	N	N	N	N		N	N
Calculus	-	-	-	-	-	-	1	-	-
STOMACH	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
 DAYS: 25-39

GROUP: IX-R      SEX: MALE  
 FATES: SCHEDULED SACRIFICE

ANIMAL ID:	164	165	166	167	168	169	170	171	172
KIDNEYS	N	N	N	N	N	N	N	N	N
ADRENALS	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*					U	*	*
Hyperplasia	-	-	-	2	2	2	-	-	-
Pigment	-	-	1	3	2	3	-	-	-

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234      GROUP: X      SEX: MALE  
 DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	174	175	176	220	221	222	274	275	276	318
NOSE	N	N	N	N			N	N	N	
Hyperplasia,goblet cell	-	-	-	-	1	1	-	-	-	1
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN				N	U	U	N		N	N
Granuloma	3	3	3	-	-	-	-	2	-	-
LUNGS										
Inflammation	2	2	1	1	1	2	-	1	-	2
Inflammation,granulomatous	2	2	2	3	3	3	2	2	2	3
Lymphoid tissue,granuloma	2	2	1	2	-	2	-	-	-	-
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N		N	N	N	N	N	N	N	N
Calculus	-	1	-	-	-	-	-	-	-	-
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: X

SEX: MALE

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FATES: SCHEDULED SACRIFICE

ANIMAL ID:	174	175	176	220	221	222	274	275	276	318
KIDNEYS	N	N	N	N	N	N	N	N	N	N
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*			*	*	*	*
Granuloma	-	-	-	-	4	2	-	-	-	-

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 20

PROJECT ID: L06234      GROUP: X      SEX: MALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	174	175	176	220	221	222	274	275	276	318
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OTHER TISSUES AND LESIONS:

THYMUS: Normal

-	-	-	-	-	-	P	-	-	-	-
---	---	---	---	---	---	---	---	---	---	---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 21

PROJECT ID: L06234  
DAYS: 25-39

GROUP: X  
FATES: SCHEDULED SACRIFICE

SEX: MALE

ANIMAL ID:	319	320
NOSE		
Hyperplasia,goblet cell	1	1
LARYNX	N	N
TRACHEA	N	N
PULMONARY LN		
Granuloma	3	3
LUNGS		
Inflammation	1	2
Inflammation,granulomatous	2	3
Lymphoid tissue,granuloma	-	3
HEART	N	N
URINARY BLADDER	N	N
STOMACH	N	N
LIVER	N	
Inflammation	-	1
SPLEEN	N	N



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X      SEX: MALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	319	320
KIDNEYS	N	N
ADRENALS	N	N
MEDIASTINAL LN	*	
Granuloma	-	4

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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**Tabulated Animal Data**

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X

SEX: MALE

FATES: SCHEDULED SACRIFICE

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ANIMAL ID:

319 320

OTHER TISSUES AND LESIONS:

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

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PROJECT ID: L06234      GROUP: X-R      SEX: MALE  
 DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	178	179	180	224	225	226	278	279	280	322
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN			U			U			U	U
Granuloma	4	3	-	4	4	-	4	4	-	-
LUNGS										
Inflammation	3	4	4	3	3	4	3	2	3	2
Inflammation,granulomatous	3	4	4	3	4	4	3	2	4	3
Lymphoid tissue,granuloma	3	3	4	4	3	4	3	1	4	3
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N		N	N	N	N	N
Calculus	-	-	-	-	1	-	-	-	-	-
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 25

PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R      SEX: MALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID	178	179	180	224	225	226	278	279	280	322
KIDNEYS	N	N	N	N	N		N	N	N	N
Nephrosis	-	-	-	-	-	4	-	-	-	-
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN				*			*		U	
Granuloma	4	4	4	-	4	4	-	3	-	4

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R      SEX: MALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	323	324
NOSE		
Hemorrhage	2	2
LARYNX	N	N
TRACHEA	N	N
PULMONARY LN	U	U
LUNGS		
Inflammation	2	2
Inflammation,granulomatous	3	4
Lymphoid tissue,granuloma	3	3
HEART	N	N
URINARY BLADDER	N	N
STOMACH	N	N
LIVER	N	N
SPLEEN	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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**Tabulated Animal Data**

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R      SEX: MALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	323	324
KIDNEYS	N	N
ADRENALS	N	N
MEDIASTINAL LN Granuloma	4	4

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: I      SEX: FEMALE  
 FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	019	020	021	022	023	024	051	052	053	054
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN	N	N	N	N	U	N	N	N	N	N
LUNGS Inflammation	-	-	3	-	-	-	-	-	1	-
HEART Inflammation, subacute	1	1	-	-	-	-	-	-	-	-
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
KIDNEYS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 29

PROJECT ID: L06234  
DAYS: 25-39

GROUP: I      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	019	020	021	022	023	024	051	052	053	054
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*	*	*	*	*	*	*



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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**Tabulated Animal Data**

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PROJECT ID: L06234      GROUP: I      SEX: FEMALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	019	020	021	022	023	024	051	052	053	054
OTHER TISSUES AND LESIONS:										
THYMUS: Normal	-	-	-	-	P	-	-	-	-	-

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: I

SEX: FEMALE

FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	055	056	091	092	093	094	095	096	139	140
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN	N	N	U	U	N	N	U	N	N	N
LUNGS		N	N	N	N	N	N	N	N	
Hemorrhage	1	-	-	-	-	-	-	-	-	-
Inflammation	-	-	-	-	-	-	-	-	-	1
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
KIDNEYS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 32

PROJECT ID: L06234  
DAYS: 25-39

GROUP: I  
SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	055	056	091	092	093	094	095	096	139	140
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*	*	*	*	*	*	*

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 33

PROJECT ID: L06234  
DAYS: 25-39

GROUP: I  
SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID: 055 056 091 092 093 094 095 096 139 140

OTHER TISSUES AND LESIONS:

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I  
FATES: SCHEDULED SACRIFICE

SEX: FEMALE

ANIMAL ID:	141	142	143	144
NOSE	N	N	N	N
LARYNX	N	N	N	N
TRACHEA	N	N	N	N
PULMONARY LN	N	N	N	N
LUNGS		N	N	
Inflammation	1	-	-	1
HEART	N	N	N	N
URINARY BLADDER	N	N	N	N
STOMACH	N	N	N	N
LIVER		N	N	N
Hepatodiaphragmatic nodule	1	-	-	-
SPLEEN	N	N	N	N
KIDNEYS	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 35

PROJECT ID: L06234      GROUP: I      SEX: FEMALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	141	142	143	144
ADRENALS	N	N	N	N
MEDIASTINAL LN	*	*	*	*

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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**Tabulated Animal Data**

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I                      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID:

141    142    143    144

OTHER TISSUES AND LESIONS:

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PAGE 37

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: I-R      SEX: FEMALE  
 FATES: SCHEDULED SACRIFICE

ANIMAL ID:	027	028	029	030	031	032	059	060	061	062
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN	N	N	N	U	N	N	N	N	N	N
LUNGS Inflammation	-	-	1	-	2	-	-	-	-	3
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
KIDNEYS	N	N	N	N	N	N	N	N	N	N



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 38

PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	027	028	029	030	031	032	059	060	061	062
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*	*	*	*	*	*	*

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R

SEX: FEMALE

FATES: SCHEDULED SACRIFICE

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ANIMAL ID:

027

028

029

030

031

032

059

060

061

062

OTHER TISSUES AND LESIONS:

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PAGE 40

PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	063	064	099	100	101	102	103	104	147	148
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN Hyperplasia	N -	N -	U -	2	2	3	2	N -	2	N -
LUNGS Inflammation	N -	N -	N -	3	2	3	2	2	1	N -
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
KIDNEYS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	063	064	099	100	101	102	103	104	147	148
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*		*	*	*	N	*	*
Hyperplasia	-	-	-	2	-	-	-	-	-	-

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID:                      063    064    099    100    101    102    103    104    147    148

OTHER TISSUES AND LESIONS:

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: Y-R  
SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	149	150	151	152
NOSE	N	N	N	N
LARYNX	N	N	N	N
TRACHEA	N	N	N	N
PULMONARY LN Hyperplasia	N -		2	N -
LUNGS Inflammation	3	2	3	1
HEART	N	N	N	N
URINARY BLADDER	N	N	N	N
STOMACH	N	N	N	N
LIVER	N	N	N	N
SPLEEN	N	N	N	N
KIDNEYS	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	149	150	151	152
ADRENALS	N	N	N	N
MEDIASTINAL LN	*	*	*	*

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:                      149      150      151      152

OTHER TISSUES AND LESIONS:

OVARY: Cyst                      -      1      -      -



PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: IX      SEX: FEMALE  
 FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	184	185	186	187	188	189	190	191	192
NOSE	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N
PULMONARY LN									
Hyperplasia	1	-	-	-	1	-	-	2	-
Pigment	1	1	2	2	2	3	2	3	2
LUNGS									
Inflammation	-	-	-	2	1	-	-	2	2
Pigment	2	3	2	3	3	2	3	3	3
HEART	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PROJECT ID: L06234  
DAYS: 25-39

GROUP: IX

SEX: FEMALE

FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	184	185	186	187	188	189	190	191	192
KIDNEYS	N	N	N	N	N	N	N	N	N
ADRENALS	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN	*	*	*	*		*	*	*	*
Hyperplasia	-	-	-	-	2	-	-	-	-
Pigment	-	-	-	-	3	-	-	-	-

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 48

PROJECT ID: L06234      GROUP: IX      SEX: FEMALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	184	185	186	187	188	189	190	191	192
OTHER TISSUES AND LESIONS:									
THYMUS: Hemorrhage	2	-	-	-	-	-	-	-	-

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

**Tabulated Animal Data**

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PROJECT ID: L06234  
 DAYS: 25-39

GROUP: X      SEX: FEMALE  
 FATES: SCHEDULED SACRIFICE

ANIMAL ID:	194	195	196	250	251	252	294	295	296	330
NOSE	N	N	N				N	N	N	
Hyperplasia, goblet cell	-	-	-	1	1	1	-	-	-	1
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN	N		N			N				N
Granuloma	-	2	-	2	3	-	3	2	2	-
LUNGS										
Inflammation	-	1	-	1	1	2	1	-	1	2
Inflammation, granulomatous	3	2	2	4	4	2	2	2	2	4
Lymphoid tissue, granuloma	-	-	-	-	-	3	-	-	-	-
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 50

PROJECT ID: L06234  
DAYS: 25-39

GROUP: X

SEX: FEMALE

FATES: SCHEDULED SACRIFICE

ANIMAL ID:	194	195	196	250	251	252	294	295	296	330
KIDNEYS	N	N	N	N	N	N	N	N	N	N
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN Hyperplasia	*	*	*	*	*	*	*	*	*	3

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PROJECT ID: L06234  
DAYS: 25-39

GROUP: X

SEX: FEMALE

FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	331	332
NOSE		N
Hyperplasia, goblet cell	1	-
LARYNX	N	N
TRACHEA	N	N
PULMONARY LN	N	
Hyperplasia	-	3
LUNGS		
Inflammation	2	3
Inflammation, granulomatous	3	2
Lymphoid tissue, granuloma	-	1
HEART	N	N
URINARY BLADDER	N	N
STOMACH	N	N
LIVER	N	N
SPLEEN	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 52

PROJECT ID: L06234      GROUP: X      SEX: FEMALE  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

ANIMAL ID:	331	332
KIDNEYS	N	N
ADRENALS	N	N
MEDIASTINAL LN	*	*

PATHOLOGY ASSOCIATES, INC.  
 FOUR WEEK INHALATION TOXICITY STUDY  
 OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
 IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PROJECT ID: L06234  
 DAYS: 25-39

GROUP: X-R      SEX: FEMALE  
 FATES: SCHEDULED SACRIFICE

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ANIMAL ID:	198	199	200	254	255	256	298	299	300	334
NOSE	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
PULMONARY LN Granuloma	4	U -	U -	U -	3	4	4	3	4	U -
LUNGS										
Inflammation	3	4	4	3	3	3	4	4	4	3
Inflammation,granulomatous	3	4	4	3	3	3	4	4	3	3
Lymphoid tissue,granuloma	4	3	3	3	3	4	3	2	-	2
HEART	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
STOMACH	N	N	N	N	N	N	N	N	N	N
LIVER		N	N	N	N	N	N	N	N	N
Hepatodiaphragmatic nodule	1	-	-	-	-	-	-	-	-	-
SPLEEN	N	N	N	N	N	N	N	N	N	N



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 54

PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	198	199	200	254	255	256	298	299	300	334
KIDNEYS	N	N	N	N	N	N	N	N	N	N
ADRENALS	N	N	N	N	N	N	N	N	N	N
MEDIASTINAL LN				*						
Granuloma	4	4	4	-	4	4	3	4	4	4

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

Tabulated Animal Data

PAGE 55

PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

ANIMAL ID:	335	336
NOSE	N	
Hemorrhage	-	2
LARYNX	N	N
TRACHEA	N	N
PULMONARY LN		U
Granuloma	4	-
LUNGS		
Inflammation	2	2
Inflammation,granulomatous	3	2
Lymphoid tissue,granuloma	1	4
HEART	N	N
URINARY BLADDER	N	N
STOMACH	N	N
LIVER	N	N
SPLEEN	N	N

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

---

Tabulated Animal Data

---

PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

PAGE 56

---

ANIMAL ID:	335	336
KIDNEYS	N	N
ADRENALS	N	N
MEDIASTINAL LN Granuloma	4	4



SECTION V

CORRELATION OF GROSS AND MICROSCOPIC (MICRO) FINDINGS

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: MALE      PAGE 1  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

---

ANIMAL ID: 003      PATHOLOGY ID. NO: 905217003      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 004      PATHOLOGY ID. NO: 905217004      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 005      PATHOLOGY ID. NO: 905217005      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 006      PATHOLOGY ID. NO: 905217006      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

---

Correlation of Gross & Micro Findings

---

PROJECT ID: L06234      GROUP: I      SEX: MALE      PAGE 2  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 007      PATHOLOGY ID. NO: 905217007      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 008      PATHOLOGY ID. NO: 905217008      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 035      PATHOLOGY ID. NO: 905217035      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LIVER, MEDIAN LOBE - MASS, 12X9X5      No corollary change detected  
MM, RED

---

ANIMAL ID: 036      PATHOLOGY ID. NO: 905217036      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: MALE      PAGE 3  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

---

ANIMAL ID: 037      PATHOLOGY ID. NO: 905217037      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 038      PATHOLOGY ID. NO: 905217038      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 039      PATHOLOGY ID. NO: 905217039      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 040      PATHOLOGY ID. NO: 905217040      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: I                      SEX: MALE                      PAGE 4  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

---

ANIMAL ID:        067                      PATHOLOGY ID. NO: 905217067    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

---

ANIMAL ID:        068                      PATHOLOGY ID. NO: 905217068    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

---

ANIMAL ID:        069                      PATHOLOGY ID. NO: 905217069    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

---

ANIMAL ID:        070                      PATHOLOGY ID. NO: 905217070    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,    No corollary change detected  
7X5X2 MM

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: 1      SEX: MALE      PAGE 5  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 071      PATHOLOGY ID. NO: 905217071      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LUNGS, BILATERAL - DIFFUSE, MOTTLED      No corollary change detected

---

ANIMAL ID: 072      PATHOLOGY ID. NO: 905217072      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 115      PATHOLOGY ID. NO: 905217115      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 116      PATHOLOGY ID. NO: 905217116      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: I                      SEX: MALE                      PAGE 6  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

---

ANIMAL ID: 117                      PATHOLOGY ID. NO: 905217117      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 118                      PATHOLOGY ID. NO: 905217118      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 119                      PATHOLOGY ID. NO: 905217119      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 120                      PATHOLOGY ID. NO: 905217120      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE      PAGE 7  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 011      PATHOLOGY ID. NO: 905217011      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>THYMUS - MOTTLED      No corollary change detected

---

ANIMAL ID: 012      PATHOLOGY ID. NO: 905217012      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LUNGS, BILATERAL - MOTTLED, PINK/RED      LUNGS- Inflammation

---

ANIMAL ID: 013      PATHOLOGY ID. NO: 905217013      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LUNGS, BILATERAL - MOTTLED, PINK/RED      LUNGS- Hemorrhage

---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: I-R                      SEX: MALE  
FATES: SCHEDULED SACRIFICE

PAGE 8

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ANIMAL ID:        014                      PATHOLOGY ID. NO: 905217014    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

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ANIMAL ID:        015                      PATHOLOGY ID. NO: 905217015    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

---

ANIMAL ID:        016                      PATHOLOGY ID. NO: 905217016    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

---

ANIMAL ID:        043                      PATHOLOGY ID. NO: 905217043    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE      PAGE 9  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 044      PATHOLOGY ID. NO: 905217044      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>THYMUS - MOTTLED, PINK/TAN. NOTE:      No corollary change detected  
ONE LOBE

---

ANIMAL ID: 045      PATHOLOGY ID. NO: 905217045      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 046      PATHOLOGY ID. NO: 905217046      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>THYMUS - MOTTLED, RED/TAN      No corollary change detected

---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: I-R                      SEX: MALE                      PAGE 10  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

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ANIMAL ID:        047                      PATHOLOGY ID. NO: 905217047    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID:        048                      PATHOLOGY ID. NO: 905217048    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

---

ANIMAL ID:        075                      PATHOLOGY ID. NO: 905217075    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID:        076                      PATHOLOGY ID. NO: 905217076    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE      PAGE 11  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 077      PATHOLOGY ID. NO: 905217077      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 078      PATHOLOGY ID. NO: 905217078      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 079      PATHOLOGY ID. NO: 905217079      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LUNGS, BILATERAL - DIFFUSE, MOTTLED      LUNGS- Inflammation  
>LYMPH NODE, BRONCHIAL - ENLARGED      No corollary change detected

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE      PAGE 12  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 080      PATHOLOGY ID. NO: 905217080      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, MEDIASTINAL - RED      MEDIASTINAL LN- Congestion

---

ANIMAL ID: 123      PATHOLOGY ID. NO: 905217123      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>THYMUS - MOTTLED, RED/TAN      THYMUS: Congestion

---

ANIMAL ID: 124      PATHOLOGY ID. NO: 905217124      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE      PAGE 13  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

---

ANIMAL ID: 125      PATHOLOGY ID. NO: 905217125      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

---

ANIMAL ID: 126      PATHOLOGY ID. NO: 905217126      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LUNGS - MOTTLED, PINK/RED      LUNGS- Inflammation

>LYMPH NODE, MEDIASTINAL - ENLARGED, 9X5X2 MM, TAN      MEDIASTINAL LN- Hyperplasia

>LIVER, MEDIAN LOBE - NODULE, 5X3X2 MM, BROWN      LIVER- Hepatodiaphragmatic nodule

---

ANIMAL ID: 127      PATHOLOGY ID. NO: 905217127      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: MALE      PAGE 14  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 128      PATHOLOGY ID. NO: 905217128      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LUNGS, BILATERAL - DIFFUSE, MOTTLED      LUNGS- Inflammation

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: IX-R      SEX: MALE      PAGE 15  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 164      PATHOLOGY ID. NO: 905217164      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Hyperplasia,  
BLACK      PULMONARY LN- Pigment

>LUNGS, BILATERAL - MOTTLED, RED/GRAY      LUNGS- Pigment

---

ANIMAL ID: 165      PATHOLOGY ID. NO: 905217165      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Hyperplasia,  
BLACK      PULMONARY LN- Pigment

>LUNGS, BILATERAL - MOTTLED, BROWN      LUNGS- Pigment

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ANIMAL ID: 166      PATHOLOGY ID. NO: 905217166      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Pigment  
6X6X2 MM, BLACK

>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Hyperplasia,  
4X4X4 MM, BLACK      PULMONARY LN- Pigment

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: IX-R      SEX: MALE      PAGE 16  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 166      PATHOLOGY ID. NO: 905217166      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LUNGS - DIFFUSE, BROWN

LUNGS- Pigment

---

ANIMAL ID: 167      PATHOLOGY ID. NO: 905217167      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
5X3X2 MM, BLACK

MEDIASTINAL LN- Hyperplasia,  
MEDIASTINAL LN- Pigment

>LUNGS - DIFFUSE, BROWN

LUNGS- Pigment

---

ANIMAL ID: 168      PATHOLOGY ID. NO: 905217168      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - BLACK

MEDIASTINAL LN- Hyperplasia,  
MEDIASTINAL LN- Pigment

>LUNGS - DIFFUSE, BROWN

LUNGS- Pigment

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: IX-R                      SEX: MALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 169                      PATHOLOGY ID. NO: 905217169      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
4X3X2 MM, BLACK

MEDIASTINAL LN- Hyperplasia,  
MEDIASTINAL LN- Pigment

>LUNGS - DIFFUSE, BROWN

LUNGS- Pigment

---

ANIMAL ID: 170                      PATHOLOGY ID. NO: 905217170      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
3X4X2 MM, BLACK

No section taken

>LYMPH NODE, BRONCHIAL - ENLARGED,  
7X4X3 MM, BLACK

PULMONARY LN- Hyperplasia,  
PULMONARY LN- Pigment

>LUNGS - DIFFUSE, BROWN

LUNGS- Pigment

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: IX-R                      SEX: MALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 171                      PATHOLOGY ID. NO: 905217171      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - DARK RED/GRAY

LUNGS- Pigment

---

ANIMAL ID: 172                      PATHOLOGY ID. NO: 905217172      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED, 4  
MM DIAMETER, BLACK

PULMONARY LN- Hyperplasia,  
PULMONARY LN- Pigment

>LUNGS - DIFFUSE, BROWN

LUNGS- Pigment

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: X                      SEX: MALE                      PAGE 19  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 174                      PATHOLOGY ID. NO: 905217174                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE                      DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, BRONCHIAL - ENLARGED,                      PULMONARY LN- Granuloma  
5X3X3 MM

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ANIMAL ID: 175                      PATHOLOGY ID. NO: 905217175                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE                      DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 176                      PATHOLOGY ID. NO: 905217176                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE                      DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, BRONCHIAL - ENLARGED,                      PULMONARY LN- Granuloma  
5X3X2 MM

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: X      SEX: MALE      PAGE 20  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 220      PATHOLOGY ID. NO: 905217220      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS, BILATERAL - MOTTLED

LUNGS- Inflammation, granulomatous

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ANIMAL ID: 221      PATHOLOGY ID. NO: 905217221      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
8X3X2 MM, ONE, TAN

MEDIASTINAL LN- Granuloma

---

ANIMAL ID: 222      PATHOLOGY ID. NO: 905217222      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>THYMUS - MOTTLED, RED/TAN

No corollary change detected

>LUNGS - MOTTLED, DARK RED/PINK

LUNGS- Inflammation, granulomatous

>LYMPH NODE, BRONCHIAL - ENLARGED,  
2X1X1 MM, ONE, TAN

No section taken

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
4X3X2 MM, ONE, WHITE

MEDIASTINAL LN- Granuloma

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: X                      SEX: MALE                      PAGE 21  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

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ANIMAL ID:        274                      PATHOLOGY ID. NO: 905217274    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID:        275                      PATHOLOGY ID. NO: 905217275    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID:        276                      PATHOLOGY ID. NO: 905217276    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:  
>LUNGS - RED, MOTTLED                      LUNGS- Inflammation,granulomatous

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ANIMAL ID:        318                      PATHOLOGY ID. NO: 905217318    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X  
FATES: SCHEDULED SACRIFICE

SEX: MALE

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ANIMAL ID: 319                      PATHOLOGY ID. NO: 905217319      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

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ANIMAL ID: 320                      PATHOLOGY ID. NO: 905217320      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Granuloma  
4X4X3 MM, ONE, TAN

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R                      SEX: MALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 178                      PATHOLOGY ID. NO: 905217178    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED,  
5X4X3 MM

PULMONARY LN- Granuloma

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
7X5X3 MM

MEDIASTINAL LN- Granuloma

>LUNGS, BILATERAL - DIFFUSE, MOTTLED

LUNGS- Inflammation,granulomatous

---

ANIMAL ID: 179

PATHOLOGY ID. NO: 905217179

PATHOLOGIST: MT

ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
12X8X3 MM, ONE, TAN

MEDIASTINAL LN- Granuloma

>LUNGS - MOTTLED, TAN/PINK

LUNGS- Inflammation,granulomatous

---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: X-R      SEX: MALE      PAGE 24  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 180      PATHOLOGY ID. NO: 905217180      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Granuloma  
10X7X5 MM, TWO, TAN

>LUNGS - MOTTLED, TAN/PINK

LUNGS- Inflammation,granulomatous

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ANIMAL ID: 224      PATHOLOGY ID. NO: 905217224      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS, BILATERAL - MOTTLED, PINK/RED      LUNGS- Inflammation,granulomatous

---

ANIMAL ID: 225      PATHOLOGY ID. NO: 905217225      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Granuloma  
9X6X5 MM

>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Granuloma  
8X5X2 MM, RED

>LUNGS - FOCI, 1X1 MM, DIFFUSE, RED

LUNGS- Inflammation,granulomatous

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PATHOLCGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R                      SEX: MALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID:        226                      PATHOLOGY ID. NO: 905217226    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - MOTTLED, PINK/GRAY/WHITE

LUNGS- Inflammation,granulomatous

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
8X4X2 MM, TWO, TAN

MEDIASTINAL LN- Granuloma

>KIDNEY, LEFT - SMALL, 10X6X5 MM

KIDNEYS- Nephrosis

---

ANIMAL ID:        278                      PATHOLOGY ID. NO: 905217278    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED

PULMONARY LN- Granuloma

>LUNGS - DIFFUSE, MOTTLED

LUNGS- Inflammation,granulomatous

---

ANIMAL ID:        279                      PATHOLOGY ID. NO: 905217279    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
9X7X2 MM

MEDIASTINAL LN- Granuloma

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: X-R      SEX: MALE      PAGE 26  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 279      PATHOLOGY ID. NO: 905217279      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Granuloma  
8X9X4 MM  
>LUNGS - MOTTLED, RED      LUNGS- Inflammation, granulomatous

---

ANIMAL ID: 280      PATHOLOGY ID. NO: 905217280      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LUNGS - MOTTLED, PINK/TAN      LUNGS- Inflammation, granulomatous  
>LYMPH NODE, MEDIASTINAL - ENLARGED,      No section taken  
9X5X4 MM, TWO, TAN

---

ANIMAL ID: 322      PATHOLOGY ID. NO: 905217322      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LUNGS - MOTTLED, TAN/RED      LUNGS- Inflammation, granulomatous  
>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Granuloma  
10X6X2 MM, TAN

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R                      SEX: MALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID:        323                      PATHOLOGY ID. NO: 905217323    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - MOTTLED, TAN/PINK

LUNGS- Inflammation,granulomatous

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
9X5X3 MM, TAN

MEDIASTINAL LN- Granuloma

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ANIMAL ID:        324                      PATHOLOGY ID. NO: 905217324    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - MOTTLED, TAN/RED

LUNGS- Inflammation,granulomatous

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
10X8X4 MM, TAN

MEDIASTINAL LN- Granuloma



PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: FEMALE      PAGE 28  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 019      PATHOLOGY ID. NO: 905217019      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 020      PATHOLOGY ID. NO: 905217020      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 021      PATHOLOGY ID. NO: 905217021      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 022      PATHOLOGY ID. NO: 905217022      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: FEMALE      PAGE 29  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 023      PATHOLOGY ID. NO: 905217023      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>THYMUS - LESION, MOTTLED, RED      No corollary change detected

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ANIMAL ID: 024      PATHOLOGY ID. NO: 905217024      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 051      PATHOLOGY ID. NO: 905217051      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 052      PATHOLOGY ID. NO: 905217052      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: FEMALE      PAGE 30  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 053      PATHOLOGY ID. NO: 905217053      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 054      PATHOLOGY ID. NO: 905217054      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 055      PATHOLOGY ID. NO: 905217055      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 056      PATHOLOGY ID. NO: 905217056      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - DARK      No corollary change detected

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: FEMALE      PAGE 31  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 091      PATHOLOGY ID. NO: 905217091      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 092      PATHOLOGY ID. NO: 905217092      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 093      PATHOLOGY ID. NO: 905217093      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 094      PATHOLOGY ID. NO: 905217094      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: FEMALE      PAGE 32  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 095      PATHOLOGY ID. NO: 905217095      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 096      PATHOLOGY ID. NO: 905217096      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 139      PATHOLOGY ID. NO: 905217139      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 140      PATHOLOGY ID. NO: 905217140      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I      SEX: FEMALE      PAGE 33  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 141      PATHOLOGY ID. NO: 905217141      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LIVER, MEDIAN LOBE - MASS, 7X5X3 MM      LIVER- Hepatodiaphragmatic nodule

---

ANIMAL ID: 142      PATHOLOGY ID. NO: 905217142      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 143      PATHOLOGY ID. NO: 905217143      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 144      PATHOLOGY ID. NO: 905217144      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE      PAGE 34  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 027      PATHOLOGY ID. NO: 905217027      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 028      PATHOLOGY ID. NO: 905217028      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 029      PATHOLOGY ID. NO: 905217029      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>EYE, RIGHT - LESION, 5X4 MM. NOTE: (Tissue unsuitable)  
MICROPHTHALMIA

---

ANIMAL ID: 030      PATHOLOGY ID. NO: 905217030      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE      PAGE 35  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 031      PATHOLOGY ID. NO: 905217031      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 032      PATHOLOGY ID. NO: 905217032      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 059      PATHOLOGY ID. NO: 905217059      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 060      PATHOLOGY ID. NO: 905217060      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE      PAGE 36  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 061      PATHOLOGY ID. NO: 905217061      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED      No corollary change detected

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ANIMAL ID: 062      PATHOLOGY ID. NO: 905217062      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 063      PATHOLOGY ID. NO: 905217063      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 064      PATHOLOGY ID. NO: 905217064      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE      PAGE 37  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 099      PATHOLOGY ID. NO: 905217099      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 100      PATHOLOGY ID. NO: 905217100      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Hyperplasia  
5X5X2 MM, RED

>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Hyperplasia  
5X4X3 MM

---

ANIMAL ID: 101      PATHOLOGY ID. NO: 905217101      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LUNGS, BILATERAL - MOTTLED      LUNGS- Inflammation

>LYMPH NODE, BRONCHIAL - ENLARGED      PULMONARY LN- Hyperplasia

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE      PAGE 38  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 102      PATHOLOGY ID. NO: 905217102      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Hyperplasia  
7X5X2 MM

---

ANIMAL ID: 103      PATHOLOGY ID. NO: 905217103      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 104      PATHOLOGY ID. NO: 905217104      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, MEDIASTINAL - ENLARGED,      No corollary change detected  
6X6X3 MM

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE      PAGE 39  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 147      PATHOLOGY ID. NO: 905217147      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, BRONCHIAL - ENLARGED      PULMONARY LN- Hyperplasia

---

ANIMAL ID: 148      PATHOLOGY ID. NO: 905217148      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 149      PATHOLOGY ID. NO: 905217149      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 150      PATHOLOGY ID. NO: 905217150      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>OVARY, LEFT - CYST, 8 MM DIAMETER      OVARY: Cyst

---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: I-R      SEX: FEMALE      PAGE 40  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 151      PATHOLOGY ID. NO: 905217151      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 152      PATHOLOGY ID. NO: 905217152      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: IX      SEX: FEMALE      PAGE 41  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 184      PATHOLOGY ID. NO: 905217184      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LYMPH NODE, BRONCHIAL - ENLARGED, 4X3X2 MM, DARK	PULMONARY LN- Hyerplasia
>THYMUS - LESION, 7X10X5 MM, RED. NOTE: HEMORRHAGE	THYMUS: Hemorrhage
>LUNGS - DARK BROWN	LUNGS- Pigment

---

ANIMAL ID: 185      PATHOLOGY ID. NO: 905217185      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNGS - GRAY/BLACK	LUNGS- Pigment

---

ANIMAL ID: 186      PATHOLOGY ID. NO: 905217186      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE      DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNGS - DARK RED/GRAY	LUNGS- Pigment

---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: IX  
SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 187                      PATHOLOGY ID. NO: 905217187    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS, BILATERAL - DARK BROWN

LUNGS- Pigment

>LYMPH NODE, BRONCHIAL - DARK BROWN

PULMONARY LN- Pigment

---

ANIMAL ID: 188

PATHOLOGY ID. NO: 905217188    PATHOLOGIST: MT

ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
6X4X2 MM, DARK

MEDIASTINAL LN- Pigment,  
MEDIASTINAL LN- Hyperplasia

>LUNGS - DARK BROWN

LUNGS- Pigment

---

ANIMAL ID: 189

PATHOLOGY ID. NO: 905217189    PATHOLOGIST: MT

ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - GRAY/BLACK

LUNGS- Pigment

>LYMPH NODE, BRONCHIAL - GRAY

PULMONARY LN- Pigment

---

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: IX      SEX: FEMALE      PAGE 43  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 190      PATHOLOGY ID. NO: 905217190      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Pigment  
4X3X2 MM, DARK

>LUNGS - DARK BROWN      LUNGS- Pigment

---

ANIMAL ID: 191      PATHOLOGY ID. NO: 905217191      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LUNGS - DARK      LUNGS- Pigment

>LYMPH NODE, BRONCHIAL - DARK      PULMONARY LN- Pigment, PULMONARY  
LN- Hyperplasia

---

ANIMAL ID: 192      PATHOLOGY ID. NO: 905217192      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:

>LUNGS - GRAY/BLACK      LUNGS- Pigment

>LYMPH NODE, BRONCHIAL - GRAY      PULMONARY LN- Pigment

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: X                      SEX: FEMALE                      PAGE 44  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 194                      PATHOLOGY ID. NO: 905217194                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:  
>LUNGS - DARK                      LUNGS- Inflammation,granulomatous

---

ANIMAL ID: 195                      PATHOLOGY ID. NO: 905217195                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:  
>SPLEEN - DEFORMITY, 5X2 MM. NOTE:                      No corollary change detected  
CONSTRICION

---

ANIMAL ID: 196                      PATHOLOGY ID. NO: 905217196                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: X                      SEX: FEMALE                      PAGE 45  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

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ANIMAL ID:        250                      PATHOLOGY ID. NO: 905217250    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:  
>LUNGS - MOTTLED                      LUNGS- Inflammation,granulomatous

---

ANIMAL ID:        251                      PATHOLOGY ID. NO: 905217251    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID:        252                      PATHOLOGY ID. NO: 905217252    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID:        294                      PATHOLOGY ID. NO: 905217294    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234                      GROUP: X                      SEX: FEMALE                      PAGE 46  
DAYS: 25-39                      FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 295                      PATHOLOGY ID. NO: 905217295                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 296                      PATHOLOGY ID. NO: 905217296                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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ANIMAL ID: 330                      PATHOLOGY ID. NO: 905217330                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,                      MEDIASTINAL LN- Hyperplasia  
6X5X2 MM

---

ANIMAL ID: 331                      PATHOLOGY ID. NO: 905217331                      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:                      RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X  
FATES: SCHEDULED SACRIFICE

SEX: FEMALE

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ANIMAL ID: 332  
ANIMAL FATE: SCHEDULED SACRIFICE

PATHOLOGY ID. NO: 905217332

PATHOLOGIST: MT

DAYS ON TEST: 25

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R                      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID: 198                      PATHOLOGY ID. NO: 905217198      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LIVER, MEDIAN LOBE - MASS, 6X5X3  
MM, RED

LIVER- Hepatodiaphragmatic nodule

>LYMPH NODE, BRONCHIAL - ENLARGED,  
8X5X3 MM

PULMONARY LN- Granuloma

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
10X8X3 MM

MEDIASTINAL LN- Granuloma

>LUNGS, BILATERAL - MOTTLED, TAN/RED

LUNGS- Inflammation,granulomatous

---

ANIMAL ID: 199

PATHOLOGY ID. NO: 905217199

PATHOLOGIST: MT

ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
6X5X2 MM, TWO, TAN

MEDIASTINAL LN- Granuloma

>LUNGS - MOTTLED, PINK-TAN

LUNGS- Inflammation,granulomatous

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: X-R      SEX: FEMALE      PAGE 49  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

---

ANIMAL ID: 200      PATHOLOGY ID. NO: 905217200      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - MOTTLED, TAN/PINK

LUNGS- Inflammation,granulomatous

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
8X5X5 MM, TWO, TAN

MEDIASTINAL LN- Granuloma

---

ANIMAL ID: 254      PATHOLOGY ID. NO: 905217254      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - MOTTLED, PINK/TAN

LUNGS- Inflammation,granulomatous

---

ANIMAL ID: 255      PATHOLOGY ID. NO: 905217255      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
9X6X3 MM

MEDIASTINAL LN- Granuloma

>LYMPH NODE, BRONCHIAL - ENLARGED,  
8X6X3 MM

PULMONARY LN- Granuloma

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R                      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

PAGE 50

---

ANIMAL ID: 256                      PATHOLOGY ID. NO: 905217256    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
9X6X5 MM

MEDIASTINAL LN- Granuloma

>LYMPH NODE, BRONCHIAL - ENLARGED,  
12X6X4 MM

PULMONARY LN- Granuloma

>LUNGS - FOCI, 1X1 MM, WHITE, DIFFUSE    LUNGS- Inflammation,granulomatous

---

ANIMAL ID: 298

PATHOLOGY ID. NO: 905217298    PATHOLOGIST: MT

ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
12X7X5 MM, MOTTLED

MEDIASTINAL LN- Granuloma

>LYMPH NODE, BRONCHIAL - ENLARGED,  
5X7X3 MM

PULMONARY LN- Granuloma

PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234      GROUP: X-R      SEX: FEMALE      PAGE 51  
DAYS: 25-39      FATES: SCHEDULED SACRIFICE

---

ANIMAL ID: 299      PATHOLOGY ID. NO: 905217299      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Granuloma  
7X4X2 MM  
>LUNGS, BILATERAL - MOTTLED, GRAY      LUNGS- Inflammation,granulomatous

---

ANIMAL ID: 300      PATHOLOGY ID. NO: 905217300      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, MEDIASTINAL - ENLARGED,      MEDIASTINAL LN- Granuloma  
9X10X5 MM  
>LYMPH NODE, BRONCHIAL - ENLARGED,      PULMONARY LN- Granuloma  
8X5X5 MM

---

ANIMAL ID: 334      PATHOLOGY ID. NO: 905217334      PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE  
DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:      RELATED HISTOPATHOLOGY:  
>LYMPH NODE, MEDIASTINAL - ENLARGED      MEDIASTINAL LN- Granuloma

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PATHOLOGY ASSOCIATES, INC.  
FOUR WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS  
IITRI PROJECT NUMBER L06234, STUDY NUMBER 1

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Correlation of Gross & Micro Findings

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PROJECT ID: L06234  
DAYS: 25-39

GROUP: X-R                      SEX: FEMALE  
FATES: SCHEDULED SACRIFICE

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ANIMAL ID:        335                      PATHOLOGY ID. NO: 905217335    PATHOLOGIST: MT  
ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
11X8X2 MM

MEDIASTINAL LN- Granuloma

>LYMPH NODE, BRONCHIAL - ENLARGED,  
6X6X3 MM

PULMONARY LN- Granuloma

---

ANIMAL ID:        336

PATHOLOGY ID. NO: 905217336    PATHOLOGIST: MT

ANIMAL FATE: SCHEDULED SACRIFICE

DAYS ON TEST:39

REFERENCE TO NECROPSY RECORD:

RELATED HISTOPATHOLOGY:

>LUNGS - MOTTLED, TAN/PINK

LUNGS- Inflammation,granulomatous

>LYMPH NODE, MEDIASTINAL - ENLARGED,  
9X4X3 MM, TAN

MEDIASTINAL LN- Granuloma



Pathology Report  
IIT Research Institute  
L06234, Study Number 1

SECTION VI  
QUALITY ASSURANCE STATEMENT

## QUALITY ASSURANCE STATEMENT

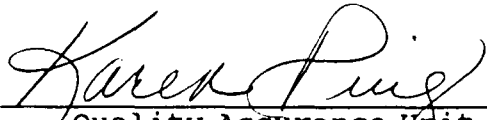
This histopathology project was inspected and audited by the PAI Quality Assurance Unit (QAU) as required by the Good Laboratory Practice (GLP) regulations promulgated by the U.S. Food and Drug Administration. Results of these activities indicate that the portions of the study performed by PAI conformed with GLP regulations and applicable Standard Operating Procedures. The pathology narrative report is an accurate reflection of the recorded data. The following table is a record of the inspections/audits performed and reported by the QAU:

Date of Inspection	Phase Inspected	Date Findings Reported to Management and Study Pathologist
**12-Nov-90	Tissue Trimming	12-Nov-90
*25-Jan-91	Processing/Embedding	30-Jan-91
*25-Jan-91	Microtomy	30-Jan-91
*06-Feb-91	Staining	06-Feb-91
*06-Feb-91	Coverslipping	06-Feb-91
*11-Feb-91	Labeling	11-Feb-91
*05-Feb-91	Quality Control/Checkout	06-Feb-91
**05-Mar-91	Individual Animal Data	13-Mar-91
**05-Mar-91	Data Entry	13-Mar-91
**07-Mar-91	Computer Validation	13-Mar-91
**12-Mar-91	Pathology Report dated 3/11/91	13-Mar-91

\*General quarterly phase inspection

\*\*Inspection specific for Study Number

In accordance with the PAI Quality Assurance Division's Standard Operating Procedures, all critical phase inspections are conducted on a random basis quarterly or more frequently. Those general phase inspections listed are the most recent conducted during the period each task associated with this project was performed.

  
\_\_\_\_\_  
Quality Assurance Unit  
PAI Illinois Division

13-Mar-91

\_\_\_\_\_  
Date

Four Week Inhalation Toxicity Study of a Solid Particulate Aerosol  
in F344/N Rats, Project Number L06234, Study Number 1

PART ONE

APPENDIX C: STUDY PROTOCOL

IIT RESEARCH INSTITUTE

Inhalation Toxicity of Single Materials  
and Mixtures, Phase II.  
Contract No. DAMD A7-89-C-9043  
IITRI Project No. L06234, Study No. 1

PROTOCOL

I. Study Title: FOUR-WEEK REPEATED DOSE INHALATION TOXICITY STUDY WITH AEROSOLS OF A SOLID PARTICULATE TEST MATERIAL IN MALE AND FEMALE F344/N RATS TO EVALUATE THE EFFECTS OF EXPOSURE CONCENTRATION, DURATION, FREQUENCY, AND RECOVERY TIME ON VARIOUS BIOLOGICAL ENDPOINTS

II. Sponsor and Sponsor's Representative:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
JACK DACRE, PH.D., CONTRACTING OFFICER'S TECHNICAL  
REPRESENTATIVE (COTR)  
FORT DETRICK  
FREDERICK, MARYLAND 21701-5012

III. Testing Laboratory:

IIT RESEARCH INSTITUTE (IITRI)  
LIFE SCIENCES RESEARCH DEPARTMENT  
10 WEST 35th STREET  
CHICAGO, ILLINOIS 60616

IV. Protocol Approval:

1. Study Director: Jeannie Bradof Date: 9/17/90  
Jeannie Bradof
2. Principal Investigator: Catherine Aranyi Date: 9/17/90  
Catherine Aranyi
3. Quality Assurance Manager: Ronald Boyne Date: 9-17-90  
Ronald Boyne
4. Sponsor (COTR): Jack F. Dacre Date: 9/28/90  
Dr. Jack Dacre

IIT RESEARCH INSTITUTE

V. Objective

The objective of this inhalation exposure study to aerosols of a solid particulate test material is to evaluate the effects of aerosol exposure concentration, daily duration, and weekly frequency on selected biologic endpoint parameters; to establish if sex of the rats is affecting the data; and to evaluate the impact of a recovery period on the results. For the overall experimental and statistical evaluation of the study, a fractional factorial design, which allows for the most efficient use of resources, will be used. The four-week exposure study will be conducted with male and female F344/N rats. Biological endpoints will include pulmonary lavage (cell number and type, phagocytic function, protein content of the lavage fluid), pulmonary function, lung wet/dry weights, and histopathology/clinical pathology. These endpoints will be evaluated within 24 hrs after the last exposure and after a 2-week recovery period. In addition, all animals on test will be monitored for in-life clinical signs, body weights, and food consumption (selected groups) throughout the study.

VI. Route of Exposure: Whole body inhalation exposure

VII. Proposed Schedule of Events

<u>EVENT</u>	<u>DATE(S)</u>
Report for Test Atmosphere Generation and Monitoring Method Development:	02/01/90
Animal Receipt:	week of 09/17/90
Quarantine Period:	receipt (week of 09/17/90) - 09/30/90
Quarantine Necropsy and Pre-exposure Health Examination:	09/28/90
Randomization and Identification:	09/27-28/90
Exposure Start: (see details in Section XII)	10/01-04,08-11/90
Body Weights and Clinical Observations: (see details in Section XII)	10/01-11/01/90 11/05-11/08/90 11/12-11/15/90

Mortality Observations:	Daily 10/01-11/18/90
Exposure Termination:	10/24,25,31,11/1,2,3/90
Post-Exposure Assays (preceded by final body weights):	
Lavage Parameters:	10/25-26/90, 11/01-02/90
Pathology/Clin. Path.:	10/25-26/90, 11/01-02/90
Pulmonary Function:	11/01-04/90
Post-Recovery Assays (preceded by final body weights):	
Lavage Parameters:	11/08-09/90, 11/15-16/90
Pathology/Clin. Path.:	11/08-09/90, 11/15-16/90
Pulmonary Function:	11/15-18/90

VIII. Test Article:

1. Identification:

a. Test Article: Powder Al

Shipping Code No. W23HYY-9233-3001

b. Positive Control Test Material:

Cristobalite, crystalline silica, 400 mesh

2. Receipt:

The test article was supplied by the Sponsor (U.S. Army, Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD) and shipped from Aberdeen Proving Ground, MD. Two fiber board drums containing a total of 95 lb were received on August 31, 1989. Approximately 10 lb of the positive control material (cristobalite) was procured from C.E.D. Process Minerals, Inc., Gore, VA 22637 and received on March 31, 1989.

3. Storage:

The test article and the positive control material will be stored in the original shipping containers at ambient room temperature (approx. 24°C) in a secure area.

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4. Assay:

The sponsor will provide purity data of the test article.

5. Handling and Hazards to Personnel:

Personnel will wear a laboratory smock, latex gloves, a respirator fitted with HEPA filter cartridges, and eye protection when working with the neat test article, unless other information is provided by the Sponsor.

6. Disposition:

All quantities of test article which are dispensed will be documented (SOP: GT-201R1). At the time of the acceptance of the final report by the Sponsor, arrangements will be made for proper disposal or the return of residual test article to the Sponsor; IITRI will not be required to retain any samples. A reserve sample will be sent to the Sponsor upon initiation of the study and retained by the Sponsor.

IX. Inhalation Exposure:

1. Inhalation Exposure Laboratories:

The supply air for the laboratories and inhalation exposure chambers is preconditioned and automatically controlled with a thermostat and humidistat to maintain the required temperature and humidity ranges (23-27°C and 40-70%RH). The single-pass conditioned room air is introduced into the chambers through individual inlet filter assemblies consisting of a coarse filter and a HEPA filter.

The exhaust from the individual aerosol exposure chambers passes through a bag-type large surface area pre-filter, followed by a HEPA filter and connected by flexible ducting to a common 20 cm diameter duct. The combined exhaust is moved by a pressure blower and exhausted outside the building. Each chamber airflow is individually controlled with a gate valve and monitored with a calibrated orifice meter located downstream of the filter. Exhaust filter loading is monitored by determining pressure drop across the filters with a magnahelic pressure differential gauge.

The inhalation chambers will be operated at an air flow rate of 500 liters per minute and maintained at a pressure that is negative to the laboratory environment.

The chambers used for the control animals are located in a separate laboratory with an exhaust system which is independent of the experimental test chambers.

## 2. Daily Exposure Schedule and Chamber Assignment:

The study will be conducted in a total of seven  $\text{m}^3$  inhalation exposure chambers. The test article aerosol target concentrations will be 100 (C1), and 200 (C2)  $\text{mg}/\text{m}^3$ ; the Cristobalite (positive control) aerosol target concentration will be 200 (CP)  $\text{mg}/\text{m}^3$ . Control animals will be exposed to filtered air (CO). Exposures will be conducted for 1 hr (D1) or 4 hr (D2) /day and exposure frequencies will be two (F1) or four (F2) times per week.

## 3. Test Atmosphere Generation:

### 3.1 The Particulate Aerosol Generator:

The test article aerosol generation system employed in these studies was developed at Oak Ridge National Laboratories (ORNL) and consists of a compressed air-driven jetmill dispersing unit fed by a screw feeder which controls the delivery rate of the test article bulk material. The generator is interfaced to the chamber air supply through a glass tee which removes large particles. The fine particles are transported to the chamber aerosol inlet. Individual aerosol generation systems are used for each inhalation exposure chamber. An identical aerosol generation system is also used to generate test atmospheres of positive control material.

## 4. Test Atmosphere Monitoring:

### 4.1 Aerosol Mass Concentration:

The aerosol mass concentration in each chamber will be determined by collecting the aerosol on 47 mm diameter glass-fiber filters placed in a close-faced plastic filter holder and pre-weighed with an analytical balance. The samples will be collected at a constant flow rate using a positive displacement diaphragm-type vacuum air pump. A dry gas meter connected to the positive pressure side of the pump will be used to record the corresponding total volume of chamber air sampled. Samples from individual chambers will be collected at a minimum of once per hour during each animal exposure period (MBIH-2R2).

In addition, aerosol mass concentration will be continuously monitored in each chamber with the in situ design backscattering photosensors (SOP: MBIH-7R1) and/or the portable continuous aerosol monitor (PCAM) real-time sensor (SOP: NTP 520-R2). At the outlet of the generator, back-scattering photosensors will be used for determining generator operation. These sensors will be employed as real-time indicators of short term changes in aerosol concentration and used in guiding laboratory personnel in making necessary adjustments in generator settings if concentration excursions are encountered.

#### 4.2 Aerosol Particle Size:

Aerosol particle size distribution will be monitored in each of the chambers except the positive control chamber with a Quartz Crystal Microbalance (QCM)-based cascade impactor. In the positive control chamber, the particle size will be monitored with a Mercer seven-stage cascade impactor. Determination of aerosol particle size distribution will be conducted during the first week of the study (SOP: MBIH-8R1).

#### 4.3 Oxygen Monitoring:

A commercial solid state oxygen analyzer will be used for monitoring percentage of oxygen in the chamber atmosphere. Oxygen concentration in each chamber will be determined in the first week of the animal exposures (SOP: MBIH-11).

#### 4.4 Temperature and Humidity:

Temperature and percent relative humidity (RH) in the individual chambers and laboratory will be monitored manually and recorded during each exposure period with a portable hand-held thermohygrometer.

### X. Animals:

#### 1. Justification/Source:

The F344/N rat was chosen for this study since it is a well characterized, sensitive test strain with a considerable amount of toxicologic background data available. The animals will be obtained from Taconic Farms Inc., Germantown, NY. Two hundred forty male and 240 female F344/N rats will be used for this study. The animals will be 4-5 weeks of age at arrival and the males will weigh approximately 55-85 grams and the females 55-75 grams.

## 2. Caging/Housing:

The inhalation studies will be conducted using 1-m<sup>3</sup> Rochester-type inhalation exposure chambers. The test animals will be housed in suspended stainless steel wire-mesh inhalation cages on mobile racks. Each mobile rack holds 24 cage units and each cage unit contains four individual cubicles for a total capacity of 96 animals per rack. Each cubicle measures 18.4 x 16.5 x 15.9 cm. The animals will be double-housed upon arrival to help them become acclimated to their new surroundings and to help them learn to use the automatic watering system. The animals will be housed individually at the time of making group assignments and will remain individually housed throughout the course of the study.

During the quarantine period and during non-exposure periods, the animals will be maintained in animal rooms located directly across the corridor from the exposure laboratories. These animal rooms and exposure laboratories are located on a semi-isolated corridor. Following randomization, the control group animals will be maintained in a separate animal room for the duration of the study. Each day just prior to the exposure periods, the animals will be transferred from the animal rooms to the exposure laboratories by removing the cage units from the mobile racks and placing them in the exposure chambers. The animals will be returned to their respective mobile racks and transferred back to the animal rooms following the exposure periods.

## 3. Animal Room/Laboratory Environmental Conditions:

The environmental conditions in the animal rooms and the exposure laboratories will be monitored and recorded daily. The lighting cycle in the animal rooms will be maintained on a 12 hour light/dark cycle. Due to the inherent design of the aerosol generator (information obtained at Contractor's Meeting), it is anticipated that chamber temperatures may range from 24 to 27°C. Therefore, the ambient laboratory and animal room temperatures and relative humidities will be regulated to avoid placing additional stress on the test animals due to extreme fluctuations between the exposure chambers and the laboratories and animal rooms respectively. The conditioned air supplied to the animal rooms and exposure laboratories is 100% fresh filtered air. The exhaust air will be cleaned via HEPA filtration prior to releasing into the atmosphere. Air flow in the animal rooms will provide at least 10 complete air changes per hour, and this can be increased if it is determined that post-exposure aerosol concentrations pose a problem. We anticipate that this may occur due to the test article adhering to the animals' hair coats and to the surfaces of

the cage units. The inhalation chambers will be operated at an air flow rate of 500 liters per minute and maintained at a pressure that is negative to the laboratory environment.

4. Cleaning and Sanitation:

Prior to the animals' arrival at IITRI, the exposure laboratories, exposure chambers, mobile racks and cages, and the animal rooms will be cleaned and sanitized according to SOP: NTP-315R3, -317R1, -322R4, and -323. The exposure chambers, cage units, mobile racks, feeders, and excrement pans will be cleaned and sanitized at least weekly thereafter except during quarantine when it will occur bi-weekly. The floors of the animal rooms and laboratories will be mopped daily during the course of the study and at least twice weekly during the quarantine and recovery periods.

The automatic watering system delivery lines are flushed automatically every eight hours for a period of 15 minutes per flush cycle to ensure a continuous supply of fresh potable drinking water. Water samples will be obtained prior to the animals' arrival and submitted for microbiological evaluation.

5. Cage Boards:

Deotized animal cage boards (Shepherd Specialty Papers, Inc., Kalamazoo, MI) will be placed in the excrement pans below the cage units to absorb liquid waste during non-exposure periods. The boards will be changed at least 3 times per week.

6. Food:

Food will be Purina Certified Rodent Chow No. 5002 which will be analyzed by the manufacturer for contaminants and nutrient levels. The diet will be available ad libitum during nonexposure hours only. Food analysis reports, provided by the manufacturer, will be maintained with the study records. The food will be stored on pallets in a well ventilated room and will be used for no more than 180 days post-milling. No known contaminants are expected to be present in the feed at levels known to be capable of interfering with the conduct of the study.

7. Water:

The mobile racks and animal rooms will be equipped with automatic watering systems. The drinking water supplied will be filtered City of Chicago drinking water. The water will be available ad libitum during non-exposure periods only. The watering system will be checked daily

to ensure proper functioning. Copies of the bimonthly results of the City of Chicago water analysis will be maintained with the study records and will be included in the final report. No known contaminants are expected to be present in the water at levels known to be capable of interfering with the conduct of the study.

## 8. Animal Receipt and Quarantine:

### 8.1 Receipt and Housing:

The exterior surfaces of the shipping containers will be examined for damage, and if accepted, wiped down with a dilute solution of sodium hypochlorite as the containers are introduced into the corridor of the inhalation facility. The animals will be taken directly from their shipping containers and housed in the wire mesh inhalation cages (SOP: NTP-302R2).

### 8.2 Observation:

During this period, the animals will be observed at least daily for signs of disease such as general unthriftiness, poor hair coat, discharges from body orifices, abnormal feces, etc. Sixty animals per sex will be weighed within 48 hr of receipt. Within three working days of receipt, the animals will be evaluated by a laboratory animal veterinarian. Following randomization, two animals per sex will be selected from the excess stock for quarantine sacrifice. These animals will be bled for a standard rat virus profile (Microbiological Associates, Rockville, MD) followed by a gross necropsy. Lesions discovered during the necropsy may be evaluated by submitting appropriate samples for microbiological culture and identification and/or tissues for histological examination. The total time for the quarantine period will be from receipt to the day prior to exposure initiation for the first group (see Section VII).

## 9. Identification:

All rats used in the study will be identified by tail tattoo representing a unique number within the population making up the study. The raw data records and specimens will also be identified by the unique test animal number.

The animal identification numbers and cage assignments for the study are shown in Attachments A and B.

10. Randomization:

Rats will be assigned to groups prior to exposure initiation using a stratified weight method whereby animals are ranked in order, by weight, and assigned to study groups in random order (SOP: GT-315).

XI. Experimental Design:

1. Overview:

The study consists of five factors, three primary (concentration, duration and frequency) and two secondary (sex and recovery). The three experimental factors describing the exposure conditions which will be used at two levels each, are: concentration (C1 and C2), daily duration (D1 and D2), and weekly frequency (F1 and F2). For rats exposed to aerosols of the test article, there will be eight combinations of these exposure conditions. In addition, control rats will be exposed to filtered air (C0) or a positive control particle (CP) under the "maximal stress" conditions, i.e., F2D2. Thus, there will be a total of 10 treatment groups: eight exposed to the test aerosol at various combinations of C, F, and D and two control groups.

C1F1D1; C2F1D1; C1F2D1; C2F2D1; C0F2D2; CPF2D2;

C1F1D2; C2F1D2; C1F2D2; C2F2D2

Because of the physical limitations (equipment and personnel) of the experimental logistics that require the simultaneous testing of such a large number of conditions under a rigorous schedule, a fractional factorial statistical design was selected (The experimental design for this study was prepared in cooperation with Dr. R. Gibbons, biostatistical consultant according to our contractual agreement with the government. For details on statistical methodology, refer to the technical proposal for this contract). The fractional factorial design allows us to evaluate all possible main effects and interactions of our three primary factors and all two-way interactions involving sex and recovery. This design, in conjunction with statistical power computations based on historical data, allows us to use relatively low group sizes in combination with an "experimental endpoint day" strategy limited to 4 days after the last exposure and 4 days after the recovery period.

More specifically, for the end points of pulmonary lavage and pulmonary function, there will be three rats of each sex distributed among the various treatment conditions to the test aerosol (one set of conditions per sex). In

addition, two filtered air control rats per sex and one positive control aerosol-exposed rat per sex will be used per endpoint each day. For the histopathology/clinical pathology group we are going to use three times as many animals, i.e., we will have nine rats per sex for the aerosol treatment conditions, six per sex for air controls, and three per sex for positive controls.

Thus for each of the eight "C F D" conditions for test article aerosol exposures, there will be 15 designated rats/sex (3 lavage + 3 pulmonary function + 9 pathology) for the terminal as well as the recovery timepoints; for COD2F2, 40 rats/sex/timepoint, and for CPD2F2, 20 rats/sex/timepoint.

The total number of animals used for the study can be computed as follows:

- o 240 test aerosol-exposed rats will be distributed into four chambers (Chamber Nos. 1, 2, 3, and 5)
- o 80 positive control rats will be exposed in one chamber (Chamber No. 4)
- o 160 filtered air control rats will be distributed into two chambers (Chamber Nos. 6 and 7)

## 2. Specific Plan:

Groups consisting of 15 male and 15 female F344/N rats will be exposed to 100 (C1) or 200 (C2) mg/m<sup>3</sup> aerosols of the test article for 1 hr (D1) or 4 hr (D2)/day, two (F1) or four (F2) days/week for four weeks. A control group of 80 male and 80 female F344/N rats will be exposed to filtered air (CO) and a positive control group of 40 male and 40 female F344/N rats<sub>3</sub> will be exposed to crystalline silica aerosols at 200 mg/m<sup>3</sup> (CP) for 4 hr/day (D2), 4 days/week (F2) for 4 weeks (Table 1). The four week exposure period will be staggered over five weeks. Biological endpoints will be determined within 24 hr after the last exposure (EXP) and after a 2-week recovery period (REC). Exposure start dates will be staggered over a two-week period to accommodate the four assay days needed at the EXP and REC timepoints. Exposures in the fourth week of exposures will be shifted for some groups to accommodate the assay dates (See Attachment B for details). Biological endpoint assays for two Exposure Classes (from classes II through IX) and controls (Exposure Classes I and X) will be conducted on each endpoint day. Details of the exposure and assay dates for the different endpoints and the animal numbers to be assigned for each group are summarized in Attachment B.



Table 1. Experimental Design<sup>a</sup>

Expo. Class Code	Expo. Cons.	Expo. hr/day	Expo. freq.	Total Number of Animals	In-life Testing						EXP (Post-Exposure) Assays <sup>b</sup>						REC (Post-Recovery) Assays <sup>b</sup>					
					Daily Obs		Clinical Signs		Body Wt.		Food Consumption		LAV <sup>c</sup>		PF <sup>d</sup>		LAV <sup>e</sup>		PF <sup>f</sup>		PATH <sup>g</sup>	
					H	F	H	F	H	F	H	F	H	F	H	F	H	F	H	F	H	F
I	0	4	4	80	80	80	80	80	80	80	24	24	8	8	8	8	8	8	8	8	24	24
II	C1	1	2	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
III	C2	1	2	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
IV	C1	1	4	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
V	C2	1	4	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
VI	C1	4	2	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
VII	C2	4	2	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
VIII	C1	4	4	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
IX	C2	4	4	15	15	15	15	15	15	15	9	9	3	3	3	3	0	3	0	3	0	9
X	P	4	4	40	40	40	40	40	40	40	12	12	4	4	4	4	12	12	4	4	4	12

<sup>a</sup> Numbers represent sample sizes.

<sup>b</sup> Four assay days occur at each timepoint (EXP = within 24 hr of the last exposure; REC = 14 days after the last exposure) to accommodate all of the treatment groups. Two experimental groups plus positive and negative controls are scheduled for each assay day.

<sup>c</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles.

<sup>d</sup> Twice/day for mortality and morbidity.

<sup>e</sup> Twice weekly.

<sup>f</sup> Twice weekly for the four-week exposure period, weekly during the subsequent two-week recovery period (where applicable) and immediately before termination.

<sup>g</sup> Weekly for selected animals.

<sup>h</sup> LAV = Pulmonary lavage assays (see text).

<sup>i</sup> PF = Pulmonary function assays (see text).

<sup>j</sup> PATH = Histopathology/clinical pathology assays (see text).

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## XII. In-Life and Terminal Observations:

### 1. Core Study (All animals):

#### 1.1 Body Weights:

The body weights will be measured and recorded individually to the nearest whole gram. The body weights will be measured at study initiation, twice weekly during the four-week exposure periods, on a weekly basis during the subsequent recovery periods (see Table 2), and immediately before termination. The weighings will be in the morning prior to exposure. Body weight gains will be calculated as change relative to the body weight measurement taken prior to the first exposure.

TABLE 2

#### WEIGHING DATES

Relative Study Day <sup>a</sup> for Weighing	Exposure/Control Start Groups <sup>b</sup> and Weighing Dates <sup>c</sup>							
	S1	S2	S3	S4	S5	S6	S7	S8
1	10/01 <sup>d</sup>	10/02 <sup>d</sup>	10/03 <sup>d</sup>	10/04 <sup>d</sup>	10/08 <sup>d</sup>	10/09 <sup>d</sup>	10/10 <sup>d</sup>	10/11 <sup>d</sup>
4	10/04	10/05	10/06	10/07	10/11	10/12	10/13	10/14
8	10/08	10/09	10/10	10/11	10/15	10/16	10/17	10/18
11	10/11	10/12	10/13	10/14	10/18	10/19	10/20	10/21
15	10/15	10/16	10/17	10/18	10/22	10/23	10/24	10/25
18	10/18	10/19	10/20	10/21	10/25	10/26	10/27	10/28
22	10/22	10/23	10/24	10/25	10/29	10/30	10/31	11/01
25	10/25	10/26	10/27	10/28	11/01	11/02	11/03	11/04
29	10/29	10/30	10/31	11/01	11/05	11/06	11/07	11/08
36	11/01	11/02	11/03	11/04	11/08	11/09	11/10	11/11

<sup>a</sup> Because of staggered exposure starts, weighing days for each group are scheduled relative to its start date.

<sup>b</sup> Refer to exposure start dates in Attachment B.

<sup>c</sup> S1 to S8 refer to the eight exposure (including test article, positive control, and filtered air control) start groups. All dates are in 1990.

<sup>d</sup> Exposure start day and first weighing date.

## 1.2 Clinical Observations:

All animals will be observed twice each exposure and non-exposure day, at least six hours apart (prior to 10:00 AM and after 2:00 PM) for moribundity and mortality (SOP: NTP-420R3). Each animal will be formally examined twice weekly, once in the morning (relative study days 1, 8, 15, 22, 29, 36) and once in the afternoon (relative study days 4, 11, 18, 25, 32, 39), for clinical signs of pharmacologic and toxicologic effects of the exposure (SOP: GT-401R1). On exposure days the first mortality/moribundity observation will coincide with food removal and chamber loading operations.

The study toxicologist, or veterinarian, will visit the laboratory at least once a week to confirm, correct or expand the clinical observations. An animal whose condition suggests that it may not survive until the next observation based upon established criteria (SOP: NTP-409R4) will be euthanized and necropsied immediately, with the tissues retained in formalin. Gross lesions found at necropsy will be documented and the affected tissues processed for histopathological examination.

## 1.3 Food Consumption:

Average food consumption for each animal in the PATH "recovery" groups will be measured for one three-day and one four-day session each week during the four exposure and two recovery weeks of the study. A measured amount of food will be offered to the rats on the same days that body weights are scheduled for the exposure portion of the study (or on the first day and fourth days of the first or second week of the recovery period). On the next scheduled body weighing day (or on the fourth day of the week during the recovery period), the remaining food will be weighed and replaced with another measured amount of food. The food consumption will be calculated from the measured differences between the provided and recovered food quantities.

## 2. Pathology/Clinical Pathology (PATH):

### 2.1 Blood Collection and Clinical Pathology Examinations:

Blood samples will be collected and analyzed from the pre-designated PATH rats for the list of hematology and clinical chemistry assays shown in Table 3. Samples will be collected and analyzed on the same day. Samples will not be collected from any moribund animals in the pre-designated PATH evaluation groups. Blood collection and analysis will be conducted in a random order (SOP: NTP-

TABLE 3. LIST OF CLINICAL PATHOLOGY ASSAYS

---

Hematology Assays

Erythrocyte Count (SOP: CP 709)  
Mean Corpuscular Volume (SOP: CP 709)  
Mean Corpuscular Hemoglobin (Derived) (SOP: CP 709)  
Mean Corpuscular Hemoglobin Concentration (Derived)  
(SOP: CP 709)  
Hemoglobin (SOP: CP 709)  
Hematocrit (Derived) (SOP: CP 709)  
Erythrocyte Morphologic Assessment (SOP: CP 745.1R1)  
Leukocyte Count (SOP: CP 709)  
Leukocyte Differential Count: (SOP: CP 745R1)  
Reticulocyte Count (SOP: CP 760R3) - if anemia is present  
Platelet Count and Morphologic Assessment  
(SOP: CP 709 and SOP: CP 745.1R1)

Clinical Chemistry Assays

Total Protein (SOP: CP 644R3)  
Albumin (SOP: CP 626R3)  
Blood urea nitrogen (SOP: CP 629R3)  
Creatinine (SOP: CP 633R3)  
Alanine aminotransferase (SOP: CP 642R3)  
Alkaline phosphatase (SOP: CP 627R3)  
Creatine kinase (SOP: CP 624R2)  
Total bile acids (SOP: CP 646)  
Total cholesterol (SOP: CP 631R2)  
Glucose (SOP: CP 635R2)  
Sorbitol dehydrogenase (SOP: CP 647)  
Calcium (SOP: CP 630R3)  
Inorganic phosphate (SOP: CP 638R3)  
Triglycerides (SOP: CP 643R3)

414R3). Blood will be collected via the retroorbital sinus (SOP: NTP-419R1) under CO<sub>2</sub> anesthesia (SOP: NTP-413R3).

## 2.2 Necropsy:

All scheduled necropsies will be performed in the presence of and under the supervision of the IITRI-PAI pathologist. All unscheduled necropsies (moribund sacrifice and spontaneous deaths) will be supervised by the pathologist to the maximum extent possible, and will be performed as soon after death as possible. Animals for unscheduled necropsies will not be frozen, and every attempt will be made to refrigerate animals for no longer than eight hours prior to the necropsy. Animals will be euthanized with CO<sub>2</sub> according to SOP: NTP-421R1.

Scheduled and unscheduled necropsies, will follow the specific necropsy procedures, as described in SOP: PAI-IL-018. A complete necropsy is defined as external examination including body orifices and examination and fixation of all tissues specified for the study. Complete necropsies will be performed unless autolysis precludes all or part of the examination. In all cases, available tissue will be collected as outlined in subsequent sections of this protocol.

The tissues that will be collected and placed in fixative include the upper and lower respiratory tract (nasal turbinates, larynx, trachea, lungs, and pulmonary lymph nodes), heart, liver, spleen, kidneys, urinary bladder, stomach, and adrenals. All tissues and/or organs will be examined in situ, then dissected from the carcass, re-examined, including cut surfaces, and fixed in 10% neutral buffered formalin. Tails, used for identification purposes, will be saved in formalin.

Rats designated for scheduled necropsy and histopathologic examination will be anesthetized with carbon dioxide and exsanguinated from the abdominal aorta, either within 24 hr following the last exposure or after a two-week recovery period following the exposures. All scheduled necropsies will be initiated promptly after an animal is killed and will be performed on all animals.

## 2.3 Organ Weights:

Lung weights will be obtained at necropsy from all animals surviving until the end of the study (SOP: NTP-408R3). Lungs will be weighed to the nearest 1.0 mg. Organ weight/body weight ratios will be calculated.

## 2.4 Tissue Trimming/Histology:

Each animal will be assigned a unique histology accession number. The formalin-fixed respiratory tract tissues for all Phase II rats will be trimmed, embedded, sectioned, and stained with hematoxylin and eosin for histopathologic examination (SOP: PAI-IL-005 through -011). An expanded tissue list and additional slides will be prepared for those rats exposed to the highest dosage based on concentration and frequency, air control, and positive control animals. The Individual Animal Necropsy Form for each animal will be available for the technician at the time of trimming.

The embedding/trimming scheme for Phase II rats is shown below.

### Trimming/Embedding Scheme

- Slide 1 Nasal turbinates (3 sections)
- Slide 2 Larynx, trachea (cross and longitudinal), and pulmonary lymph nodes
- Slide 3 Lung (whole mount with all lobes presented to include the main bronchus)
- Slide 4\* Heart, stomach, urinary bladder
- Slide 5\* Liver (two lobes) and spleen
- Slide 6\* Kidneys (right and left) and adrenals (right and left)

\* These slides will be prepared only for high-dose, air control, and positive control rats.

Once all aspects of histology are completed, the residual wet tissues, blocks, and slides will be prepared for long-term archiving and/or shipment to the sponsor.

## 2.5 Histopathologic Evaluation:

Histopathologic evaluation shall be conducted on all protocol-specified tissues with findings entered into the IITRI/PAI automated pathology system (Labcat) for generation of individual animal and summary incidence tables. If significant treatment-associated tissue changes are found in the high-dose group, then the tissues from the next lower dosage group will be processed into slides and microscopically evaluated.

### 3. Pulmonary Lavage (LAV):

#### 3.1 Pulmonary Lavage, Total and Differential Cell Count:

Within 24 hr or 14 days after the last exposure, the preassigned rats from each group will be sacrificed for collection of alveolar cells by tracheobronchial lavage (SOP: MBM 2.01). Total and differential cell counts will be determined for the collected cells (SOP: MBM2.02 and MBM2.03).

#### 3.2 Pulmonary Lavage Fluid Proteins:

The supernatant from the first several lung washes will be saved for protein determination. Lavage fluids will be assayed for protein by the method of Bradford (1976) Anal. Biochem. 72:248-254 (SOP: MBM6.02M).

#### 3.3 Macrophage Fc Receptor-Mediated Phagocytosis:

The alveolar macrophages isolated from each group as described in Section XIV.3.1 will also be tested for phagocytosis of <sup>51</sup>Cr-chicken red blood cells (CRBC) using a modification of the method of Smialowicz et al. (1984) (SOP: MBM5.01R1, MBM5.02R1, and MBM5.03).

### 4. Pulmonary Function (PF):

The pulmonary function parameters will be measured using an acrylic plethysmograph equipped to allow measurement of airflow and plethysmograph, airway and esophageal pressure for anesthetized animals.

Within 24 hr (EXP) or 14 days (REC) following its last exposure, each test animal will be weighed (tattoo I.D. also recorded), anesthetized with pentobarbital, transorally tracheostomized and intubated to the distal third of the esophagus with a saline-filled polyethylene tube (SOP: T-IIT-01-00).

The following lung function determinations will be conducted (SOP: Nos. T-IIT-02-00 through T-IIT-07-00):

- 1) Resting breathing measurements: tidal volume (Vt), minute volume (Ve), frequency (f), inspiratory time (Ti), expiratory time (Te) and breathing mechanics.
- 2) Total lung capacity (TLC), vital capacity (VC) and residual volume (RV). RV is determined by Ne gas dilution.
- 3) End expiratory volume (PRC) by Boyle's law.
- 4) Multi-breath diffusing capacity of carbon monoxide (DLco).
- 5) Quasistatic compliance (Crs).
- 6) Forced expiratory maneuver (FEF 50, 25, & 10%, forced vital capacity [FVC]).

#### XIII. Statistical Methods:

A fractional factorial design will be used. According to contractual agreement with the government, the experimental design of the studies was made in cooperation with Dr. Robert Gibbons, biostatistical consultant to the program, and the statistical evaluation of the data will be conducted by him.

#### XIV. Data Notebooks:

1. Contents: All original data will be maintained in notebooks and will include but not necessarily be limited to the following:

- a. Original signed protocol and all amendments
- b. Test article information
- c. Animal receiving records
- d. Randomization procedures
- e. Animal housing and environmental conditions records
- f. Chemical generation and monitoring records
- g. Body weight records
- h. Mortality and clinical observations
- i. Food consumption records
- j. Clinical pathology records
- k. Necropsy and histopathology records
- l. Pulmonary lavage records
- m. Pulmonary function records

2. Storage:

All original data and a copy of the final report will be kept in the IITRI Archives.



XV. Final Reports:

Following review and comments by the Sponsor, the required number of copies of the final report will be submitted.

XVI. Personnel:

Curricula vitae for all IITRI personnel involved in the study are on file at IITRI.

XVII. GLP Compliance and Standard Operating Procedures:

The study will be conducted in compliance with EPA GLP requirements as specified in Part 792 of Title 40 of the Code of Federal Regulations and any later interpretations and revisions published by EPA). Quality Assurance inspections of phases of the study will be conducted as required.

This Protocol will be the controlling document, for the conduct of this study. In case of changes or discrepancies from the Protocol, these will be documented in Protocol Amendments or Deviations through which the Project Officer will be notified immediately.

# ATTACHMENT A. BOSSING POSITION MAP

BACK 1

Comp. 1

Comp. 2

1	1	2	3	4
2	5	6	7	8
3	9	10	11	12
4	13	14	15	16
5	X	X	X	X
6	X	X	X	X

7	17	18	19	20
8	21	22	23	24
9	25	26	27	28
10	29	30	31	32
11	X	X	X	X
12	X	X	X	X

BACK 2

Comp. 3

Comp. 4

25	33	34	35	36
26	37	38	39	40
27	41	42	43	44
28	45	46	47	48
29	X	X	X	X
30	X	X	X	X

31	49	50	51	52
32	53	54	55	56
33	57	58	59	60
34	61	62	63	64
35	X	X	X	X
36	X	X	X	X

13	65	66	67	68
14	69	70	71	72
15	73	74	75	76
16	77	78	79	80
17	81	82	83	84
18	85	86	87	88

19	89	90	91	92
20	93	94	95	96
21	97	98	99	100
22	101	102	103	104
23	105	106	107	108
24	109	110	111	112

37	113	114	115	116
38	117	118	119	120
39	121	122	123	124
40	125	126	127	128
41	129	130	131	132
42	133	134	135	136

43	137	138	139	140
44	141	142	143	144
45	145	146	147	148
46	149	150	151	152
47	153	154	155	156
48	157	158	159	160

X = Empty Compartment

# ATTACHMENT A. HOUSING POSITION MAP (cont'd)

RACK 3

Cage #

Cage #	49	50	51	52	53	54°
	161	165	169	173	177	201
	162	166	170	176	178	202
	163	167	171	175	179	203
	164	168	172	176	180	X

Cage #	55	56	57	58	59	60°
	181	185	189	193	197	231
	182	186	190	194	198	232
	183	187	191	195	199	233
	184	188	192	196	200	X

RACK 4

Cage #

Cage #	73	74	75	76	77	78
	361	365	369	373	377	381
	362	366	370	374	378	382
	363	367	371	375	379	383
	364	368	372	376	380	384

Cage #	79	80	81	82	83	84
	421	425	429	433	437	441
	422	426	430	434	438	442
	423	427	431	435	439	443
	424	428	432	436	440	444

Cage #	61	62	63	64	65	66
	261	265	269	273	277	X
	262	266	270	274	278	X
	263	267	271	275	279	X
	264	268	272	276	280	X

Cage #	67	68	69	70	71	72
	281	285	289	293	297	X
	282	286	290	294	298	X
	283	287	291	295	299	X
	284	288	292	296	300	X

Cage #	91	92	93	94	95	96
	X	X	X	X	X	X
	X	X	X	X	X	X
	X	X	X	X	X	X
	X	X	X	X	X	X

\* Cages 654 and 660 will be housed in cage positions 65 and 66 (in the control animal room) until their exposure start.

\* Cages 885 to 90 will be housed in cage positions 85, 11, 29-30, and 35-36 (in the control animal room) until their exposure start.

X = Empty Compartment

# ATTACHMENT A. HOUSING POSITION MAP (cont'd)

RACK 5

Comp. 1

97	204	205	206	207
98	208	209	210	211
99	212	213	214	215
100	216	217	218	X
101	219	220	221	222
102	223	224	225	226

Comp. 2

103	234	235	236	237
104	238	239	240	241
105	242	243	244	245
106	246	247	248	X
107	249	250	251	252
108	253	254	255	256

RACK 6

Comp. 1

121	305	306	307	X
122	308	309	310	X
123	301	302	303	304
124	305	306	307	308
125	309	400	401	402
126	X	X	X	X

Comp. 2

127	403	404	405	X
128	406	407	408	X
129	409	410	411	412
130	413	414	415	416
131	417	418	419	420
132	X	X	X	X

109

227	228	229	230	231
232	233	234	235	236
237	238	239	240	X
241	242	243	244	X
245	246	247	248	X
249	250	251	252	X

110

313	314	315	316	317
318	319	320	321	322
323	324	325	326	327
328	329	330	331	332
333	334	335	336	337

111

445	446	447	X	X
448	449	450	X	X
451	452	453	454	455
456	457	458	459	460
461	462	463	464	465
X	X	X	X	X

112

463	464	465	X	X
466	467	468	X	X
469	470	471	472	473
474	475	476	477	478
479	480	481	482	483
X	X	X	X	X

113

445	446	447	X	X
448	449	450	X	X
451	452	453	454	455
456	457	458	459	460
461	462	463	464	465
X	X	X	X	X

114

463	464	465	X	X
466	467	468	X	X
469	470	471	472	473
474	475	476	477	478
479	480	481	482	483
X	X	X	X	X

X = Empty Compartment

**Attachment B. Summary of Animal Numbers and Cage Locations of Rats used  
for the Various Exposure Conditions and Various Endpoints.**

Expo. Class	Expt Code <sup>a</sup>	Expt Group	Expo. Conc.	Expo. hr/day	Exposure Start <sup>b</sup>	Exposure End	Experiment Date <sup>b</sup>	Animal Numbers		Case Number(s)	
								Males	Females	Males	Females
I	LAV	0	02	F2	-	81-10/01/90	10/24/90 E1 <sup>c</sup>	1-2	17-18	1	7
I	LAV	0	02	F2	-	82-10/02/90	10/25/90 E2	33-34	49-50	25	31
I	LAV	0	02	F2	-	85-10/08/90	10/31/90 E3 <sup>c</sup>	65-66	89-90	13	19
I	LAV	0	02	F2	-	86-10/09/90	11/01/90 E4	113-114	137-138	37	43
I	PF	0	02	F2	-	85-10/08/90	11/01/90 E1P <sup>c</sup>	81-82	105-106	17	23
I	PF	0	02	F2	-	85-10/08/90	11/02/90 E2P	85-86	109-110	18	24
I	PF	0	02	F2	-	86-10/09/90	11/03/90 E3P	129-130	153-154	41	47
I	PF	0	02	F2	-	86-10/09/90	11/04/90 E4P <sup>d</sup>	133-134	157-158	42	48
I	PATN	0	02	F2	-	81-10/01/90	10/24/90 E1 <sup>c</sup>	3-8	19-24	1-2	7-8
I	PATN	0	02	F2	-	82-10/02/90	10/25/90 E2	35-40	51-56	25-26	31-32
I	PATN	0	02	F2	-	85-10/08/90	10/31/90 E3 <sup>c</sup>	67-72	91-96	13-14	19-20
I	PATN	0	02	F2	-	86-10/09/90	11/01/90 E4	115-120	139-144	37-38	43-44
IR	LAV	0	02	F2	+	81-10/01/90	10/24/90 R1 <sup>c</sup>	9-10	25-26	3	9
IR	LAV	0	02	F2	+	82-10/02/90	10/25/90 R2	41-42	57-58	27	33
IR	LAV	0	02	F2	+	85-10/08/90	10/31/90 R3 <sup>c</sup>	73-74	97-98	15	21
IR	LAV	0	02	F2	+	86-10/09/90	11/01/90 R4	121-122	145-146	39	45
IR	PF	0	02	F2	+	85-10/08/90	10/31/90 R1P <sup>c</sup>	83-84	107-108	17	23
IR	PF	0	02	F2	+	85-10/08/90	11/01/90 R2P	87-88	111-112	18	24
IR	PF	0	02	F2	+	86-10/09/90	11/02/90 R3P	131-132	155-156	41	47
IR	PF	0	02	F2	+	86-10/09/90	11/03/90 R4P <sup>d</sup>	135-136	159-160	42	48
IR	PATN	0	02	F2	+	81-10/01/90	10/24/90 R1 <sup>c</sup>	11-16	27-32	3-4	9-10
IR	PATN	0	02	F2	+	82-10/02/90	10/25/90 R2	43-48	59-64	27-28	33-34
IR	PATN	0	02	F2	+	85-10/08/90	10/31/90 R3 <sup>c</sup>	75-80	99-104	15-16	21-22
IR	PATN	0	02	F2	+	86-10/09/90	11/02/90 R4	123-128	147-152	39-40	45-46
II	LAV	C1	01	F1	-	83-10/03/90	10/24/90 E1 <sup>c</sup>	361-363	-	73	-
II	PF	C1	01	F1	-	87-10/03/90	10/31/90 E1P <sup>c</sup>	385-387	-	121	-
II	PATN	C1	01	F1	-	83-10/03/90	10/24/90 E1 <sup>c</sup>	364-372	-	73-75	-
IIIR	LAV	C1	01	F1	+	83-10/03/90	10/24/90 R1 <sup>c</sup>	-	373-375	-	74
IIIR	PF	C1	01	F1	+	87-10/10/90	10/31/90 R1P <sup>c</sup>	-	403-405	-	127
IIIR	PATN	C1	01	F1	+	83-10/03/90	10/24/90 R1 <sup>c</sup>	-	376-384	-	74-78

<sup>a</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles. R = REC (assayed after a 14 day recovery period).

<sup>b</sup> S = Exposure Start Days 1 to 8 are staggered over a two-week period; E = Assay days 1 to 4 for the EXP (within 24 hr of the last exposure) endpoint timing; R = Assay days 1 to 4 for the REC (after a 14 day recovery period) endpoint timing; P = Pulmonary function EXP and REC assays are scheduled to occur on four consecutive days each.

<sup>c</sup> For F2 and F1 groups, the fourth week of exposures will be conducted Sunday through Wednesday or Tuesday and Wednesday, respectively, to accommodate endpoint measurements on Thursday.

<sup>d</sup> For F2 and F1 PF groups, the fourth week of exposures will be conducted Wednesday through Saturday or Friday and Saturday, respectively, to accommodate endpoint measurements on Sunday.

Attachment B. Summary of Animal Numbers and Cage Locations of Rats Used for the Various Exposure Conditions and Various Endpoints.  
(Continued)

Expo. Class	Expt. Code	Expo. Group	Expo. Cond.	Expo. hr/day	Expo. Freq.	Exposure Start <sup>a</sup>	Exposure End <sup>b</sup>	Experiment Date <sup>b</sup>	Animal Numbers		Cage Number(s)	
									Males	Females	Males	Females
III	LAV	C2	D1	F1	-	38-10/11/90	11/01/90	E4 - 11/02/90	-	469-471	-	141
III	PF	C2	D1	F1	-	38-10/11/90	11/03/90	E4 <sup>d</sup> - 11/04/90	-	463-465	-	139
III	PATN	C2	D1	F1	-	38-10/11/90	11/01/90	E4 - 11/02/90	-	472-480	-	141-143
III	LAV	C2	D1	F1	+	38-10/11/90	11/01/90	R4 - 11/16/90	451-453	-	135	-
III	PF	C2	D1	F1	+	38-10/11/90	11/03/90	R4 <sup>d</sup> - 11/18/90	445-447	-	133	-
III	PATN	C2	D1	F1	+	38-10/11/90	11/01/90	R4 - 11/16/90	454-462	-	135-137	-
IV	LAV	C1	D1	F2	-	32-10/02/90	10/25/90	E2 - 10/26/90	-	281-283	-	67
IV	PF	C1	D1	F2	-	33-10/08/90	11/01/90	R2 <sup>p</sup> - 11/02/90	-	244-248	-	106
IV	PATN	C1	D1	F2	-	32-10/02/90	10/25/90	E2 - 10/26/90	-	284-292	-	67-69
IV	LAV	C1	D1	F2	+	32-10/02/90	10/25/90	R2 - 11/09/90	261-263	-	61	-
IV	PF	C1	D1	F2	+	33-10/08/90	11/01/90	R2 <sup>p</sup> - 11/16/90	216-218	-	100	-
IV	PATN	C1	D1	F2	+	32-10/02/90	10/25/90	R2 - 11/09/90	264-272	-	61-63	-
V	LAV	C2	D1	F2	-	35-10/08/90	10/31/90	E3 <sup>c</sup> - 11/01/90	204-206	-	97	-
V	PF	C2	D1	F2	-	36-10/09/90	11/02/90	E3 <sup>p</sup> - 11/03/90	307-309	-	113	-
V	PATN	C2	D1	F2	-	35-10/08/90	10/31/90	E3 <sup>c</sup> - 11/01/90	207-215	-	97-99	-
VI	LAV	C2	D1	F2	+	35-10/08/90	10/31/90	R3 <sup>c</sup> - 11/15/90	-	234-236	-	103
VI	PF	C2	D1	F2	+	36-10/09/90	11/02/90	R3 <sup>p</sup> - 11/17/90	-	310-312	-	114
VI	PATN	C2	D1	F2	+	35-10/08/90	10/31/90	R3 <sup>c</sup> - 11/15/90	-	237-245	-	103-105
VI	LAV	C1	D2	F1	-	37-10/10/90	10/31/90	E3 <sup>c</sup> - 11/01/90	-	409-411	-	129
VI	PF	C1	D2	F1	-	38-10/11/90	11/02/90	E3 <sup>p</sup> - 11/03/90	-	446-448	-	140
VI	PATN	C1	D2	F1	-	37-10/10/90	10/31/90	E3 <sup>c</sup> - 11/01/90	-	412-420	-	129-131
VII	LAV	C1	D2	F1	+	37-10/10/90	10/31/90	R3 <sup>c</sup> - 11/15/90	391-393	-	123	-
VII	PF	C1	D2	F1	+	38-10/11/90	11/02/90	R3 <sup>p</sup> - 11/17/90	448-450	-	135	-
VII	PATN	C1	D2	F1	+	37-10/10/90	10/31/90	R3 <sup>c</sup> - 11/15/90	394-402	-	123-125	-
VII	LAV	C2	D2	F1	-	34-10/04/90	10/25/90	E2 - 10/26/90	421-423	-	79	-
VII	PF	C2	D2	F1	-	37-10/10/90	11/01/90	E2 <sup>p</sup> - 11/02/90	388-390	-	122	-
VII	PATN	C2	D2	F1	-	34-10/04/90	10/25/90	E2 - 10/26/90	424-432	-	79-81	-
VIII	LAV	C2	D2	F1	+	34-10/04/90	10/25/90	R2 - 11/09/90	-	433-435	-	82
VIII	PF	C2	D2	F1	+	37-10/10/90	11/01/90	R2 <sup>p</sup> - 11/16/90	-	404-408	-	128
VIII	PATN	C2	D2	F1	+	34-10/04/90	10/25/90	R2 - 11/09/90	-	436-444	-	82-84
VIII	LAV	C1	D2	F2	-	36-10/09/90	11/01/90	E4 - 11/02/90	349-351	-	88	-
VIII	PF	C1	D2	F2	-	36-10/09/90	11/03/90	E4 <sup>d</sup> - 11/04/90	301-303	-	111	-
VIII	PATN	C1	D2	F2	-	36-10/09/90	11/01/90	E4 - 11/02/90	352-360	-	88-90	-
VIII	LAV	C1	D2	F2	+	36-10/09/90	11/01/90	R4 - 11/16/90	-	337-339	-	85
VIII	PF	C1	D2	F2	+	36-10/09/90	11/03/90	R4 <sup>d</sup> - 11/18/90	-	304-306	-	112
VIII	PATN	C1	D2	F2	+	36-10/09/90	11/01/90	R4 - 11/16/90	-	340-348	-	85-87

- <sup>a</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles. R = REC (assayed after a 14 day recovery period).
- <sup>b</sup> S = Exposure Start Days 1 to 8 are staggered over a two-week period; E = Assay days 1 to 4 for the EXP (within 24 hr of the last exposure) endpoint timing; R = Assay days 1 to 4 for the REC (after a 14 day recovery period) endpoint timing; P = Pulmonary function EXP and REC assays are scheduled to occur on four consecutive days each.
- <sup>c</sup> For F2 and F1 groups, the fourth week of exposures will be conducted Sunday through Wednesday or Tuesday and Wednesday, respectively, to accommodate endpoint measurements on Thursday.
- <sup>d</sup> For F2 and F1 groups, the fourth week of exposures will be conducted Wednesday through Saturday or Friday and Saturday, respectively, to accommodate endpoint measurements on Sunday.

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Table A1. Summary of Animal Numbers and Cage Locations of Rats used for the Various Exposure Conditions and Various Endpoints.  
(Continued)

Expo. Class	Expt. Group	Expo. Conc.	Expo. hr/day	Exposure Start <sup>b</sup>	Exposure End <sup>b</sup>	Experiment Date <sup>b</sup>	Animal Numbers		Cage Number(s)	
							Male	Female	Male	Female
IX	LAV	C2	D2	-	81-10/01/90	10/24/90	E1 <sup>c</sup>	-	101-103	-
IX	PF	C2	D2	-	85-10/08/90	10/31/90	E1P <sup>c</sup>	-	231-233	-
IX	PATN	C2	D2	-	81-10/01/90	10/24/90	E1 <sup>c</sup>	-	104-192	-
IXR	LAV	C2	D2	+	81-10/01/90	10/24/90	R1 <sup>c</sup>	-	161-163	-
IXR	PF	C2	D2	+	85-10/08/90	10/31/90	R1P <sup>c</sup>	-	201-203	-
IXR	PATN	C2	D2	+	81-10/01/90	10/24/90	R1 <sup>c</sup>	-	164-172	-
X	LAV	P	D2	-	81-10/01/90	10/24/90	E1 <sup>c</sup>	-	173-173	193-193
X	LAV	P	D2	-	82-10/02/90	10/25/90	E2	-	273-273	293-293
X	LAV	P	D2	-	85-10/08/90	10/31/90	E3 <sup>c</sup>	-	219-219	249-249
X	LAV	P	D2	-	86-10/09/90	11/01/90	E4	-	317-317	329-329
X	PF	P	D2	-	85-10/08/90	10/31/90	E1P <sup>c</sup>	-	227-227	237-237
X	PF	P	D2	-	85-10/08/90	11/01/90	E2P	-	239-239	259-259
X	PF	P	D2	-	86-10/09/90	11/02/90	E3P	-	313-313	323-323
X	PF	P	D2	-	86-10/09/90	11/03/90	E4P <sup>d</sup>	-	315-315	327-327
X	PATN	P	D2	-	81-10/01/90	10/24/90	E1 <sup>c</sup>	-	174-174	194-194
X	PATN	P	D2	-	82-10/02/90	10/25/90	E2	-	274-274	294-294
X	PATN	P	D2	-	85-10/08/90	10/31/90	E3 <sup>c</sup>	-	220-222	250-252
X	PATN	P	D2	-	86-10/09/90	11/01/90	E4	-	318-320	330-332
XR	LAV	P	D2	+	81-10/01/90	10/24/90	R1 <sup>c</sup>	-	177-177	197-197
XR	LAV	P	D2	+	82-10/02/90	10/25/90	R2	-	277-277	297-297
XR	LAV	P	D2	+	85-10/08/90	10/31/90	R3 <sup>c</sup>	-	223-223	253-253
XR	LAV	P	D2	+	86-10/09/90	11/01/90	R4	-	321-321	333-333
XR	PF	P	D2	+	85-10/08/90	10/31/90	R1P <sup>c</sup>	-	228-228	258-258
XR	PF	P	D2	+	85-10/08/90	11/01/90	R2P	-	230-230	260-260
XR	PF	P	D2	+	86-10/09/90	11/02/90	R3P	-	314-314	326-326
XR	PF	P	D2	+	86-10/09/90	11/03/90	R4P <sup>d</sup>	-	316-316	328-328
XR	PATN	P	D2	+	81-10/01/90	10/24/90	R1 <sup>c</sup>	-	178-180	198-200
XR	PATN	P	D2	+	82-10/02/90	10/25/90	R2	-	278-280	298-300
XR	PATN	P	D2	+	85-10/08/90	10/31/90	R3 <sup>c</sup>	-	224-224	254-254
XR	PATN	P	D2	+	86-10/09/90	11/01/90	R4	-	322-324	334-336

<sup>a</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles. R = REC (assayed after a 14 day recovery period).  
<sup>b</sup> S = Exposure start days 1 to 8 are staggered over a two-week period; E = Assay days 1 to 4 for the EXP (within 24 hr of the last exposure) endpoint timing; R = Assay days 1 to 4 for the REC (after a 14 day recovery period) endpoint timing; P = Pulmonary function test; and REC assays are scheduled to occur on four consecutive days each.  
<sup>c</sup> For F2 and F1 groups, the fourth week of exposures will be conducted Sunday through Wednesday or Tuesday and Wednesday, respectively, to accommodate endpoint measurements on Thursday.  
<sup>d</sup> For F2 and F1 PF groups, the fourth week of exposures will be conducted Wednesday through Saturday or Friday and Saturday, respectively, to accommodate endpoint measurements on Sunday.

Inhalation Toxicity of Single Materials  
and Mixtures, Phase II.  
Contract No. DAMD17-89-C-9043  
IITRI Project No. L06234, Study No. 1

PROTOCOL AMENDMENT 1

Study Title: **FOUR-WEEK REPEATED DOSE INHALATION TOXICITY STUDY WITH AEROSOLS OF A SOLID PARTICULATE TEST MATERIAL IN MALE AND FEMALE F344/N RATS TO EVALUATE THE EFFECTS OF EXPOSURE CONCENTRATION, DURATION, FREQUENCY, AND RECOVERY TIME ON VARIOUS BIOLOGICAL ENDPOINTS**

Effective Date: October 1, 1990

Amendment

Change: Table 2 in Section XII.1.1 is changed as follows (changes are shown in bold type).

TABLE 2

WEIGHING DATES

Relative Study Day <sup>a</sup> for Weighing	Exposure/Control Start Groups <sup>b</sup> and Weighing Dates <sup>c</sup>							
	S1	S2	S3	S4	S5	S6	S7	S8
1	10/01 <sup>d</sup>	10/02 <sup>d</sup>	10/03 <sup>d</sup>	10/04 <sup>d</sup>	10/08 <sup>d</sup>	10/09 <sup>d</sup>	10/10 <sup>d</sup>	10/11 <sup>d</sup>
4	10/04	10/05	10/06	10/07	10/11	10/12	10/13	10/14
8	10/08	10/09	10/10	10/11	10/15	10/16	10/17	10/18
11	10/11	10/12	10/13	10/14	10/18	10/19	10/20	10/21
15	10/15	10/16	10/17	10/18	10/22	10/23	10/24	10/25
18	10/18	10/19	10/20	10/21	10/25	10/26	10/27	10/28
22	10/22	10/23	10/24	10/25	10/29	10/30	10/31	11/01
25	10/25	10/26	10/27	10/28	11/01	11/02	11/03	11/04
29	10/29	10/30	10/31	11/01	11/05	11/06	11/07	11/08
36	11/05	11/06	11/07	11/08	11/12	11/13	11/14	11/15

<sup>a</sup> Because of staggered exposure starts, weighing days for each group are scheduled relative to its start date.

<sup>b</sup> Refer to exposure start dates in Attachment B.

<sup>c</sup> S1 to S8 refer to the eight exposure (including test article, positive control, and filtered air control) start groups. All dates are in 1990.

<sup>d</sup> Exposure start day and first weighing date.



Reason: Miscalculation of Relative Study Day 36 dates in original table.

Change: The first paragraph in Section XII.1.2 is changed to read as follows (changed sections are shown in bold type):

XII.1.2 Clinical Observations:

All animals will be observed twice each exposure and non-exposure day, at least six hours apart (prior to 10:00 AM and after 2:00 PM) for moribundity and mortality (SOP: NTP-420R3). Each animal will be formally examined twice weekly, once in the morning (**Mondays for Exposure Start Groups S1, S3, S5, and S7 and Tuesdays for Exposure Start Groups S2, S4, S6, and S8**) and once in the afternoon (**Thursdays for Exposure Start Groups S1, S3, S5, and S7 and Fridays for Exposure Start Groups S2, S4, S6, and S8**), for clinical signs of pharmacologic and toxicologic effects of the exposure (SOP: GT-401R1). On exposure days the first mortality/ moribundity observation will coincide with food removal and chamber loading operations.

Reason: Clarification of observation days requested by Study Toxicologist.

Change: Attachment B is changed as indicated in the attached copy (changes are shown in bold type).

Reason: Experiment dates for LAV assays E3 and E4 and Exposure End dates for PF assays E1P, E2P, E3P and E4P were incorrect in the table portion for Exposure Class Code I.

Approval:

Study Director:

Jeannie Bradof  
Jeannie Bradof

Date: 9/28/90

Principal Investigator:

Catherine Aranyi  
Catherine Aranyi

Date: 9/28/90

Quality Assurance Manager:

Ronald Boyne  
Ronald Boyne

Date: 9-30-90

Sponsor (COTR):

Dr. Jack Dacre  
Dr. Jack Dacre

Date: 10/17/90

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**Attachment B. Summary of Animal Numbers and Cage Locations of Data used  
for the Various Exposure Conditions and Various Endpoints.**

Expo. Class	Expt Code	Expt Group	Expo. Conc.	Expo. Dose	Expo. Freq.	Recovery	Exposure		Experiment Date <sup>b</sup>	Animal Numbers		Case Number(s)	
							Start	End		Males	Females	Males	Females
I	I	LAV	0	D2	F2	-	81-10/01/90	10/24/90	E1 <sup>c</sup> - 10/25/90	1-2	17-18	1	7
I	I	LAV	0	D2	F2	-	82-10/02/90	10/25/90	E2 - 10/26/90	33-34	49-50	25	31
I	I	LAV	0	D2	F2	-	85-10/08/90	10/31/90	E3 <sup>c</sup> - 11/01/90	65-66	89-90	13	19
I	I	LAV	0	D2	F2	-	86-10/09/90	11/01/90	E4 - 11/02/90	113-114	137-138	37	43
I	I	PF	0	D2	F2	-	85-10/08/90	10/31/90	E1P <sup>c</sup> - 11/01/90	81-82	105-106	17	23
I	I	PF	0	D2	F2	-	85-10/08/90	11/01/90	E2P - 11/02/90	85-86	109-110	18	24
I	I	PF	0	D2	F2	-	86-10/09/90	11/02/90	E3P - 11/03/90	129-130	153-154	41	47
I	I	PF	0	D2	F2	-	86-10/09/90	11/03/90	E4P <sup>d</sup> - 11/04/90	133-134	157-158	42	48
I	I	PATH	0	D2	F2	-	81-10/01/90	10/24/90	E1 <sup>c</sup> - 10/25/90	3-8	19-24	1-2	7-8
I	I	PATH	0	D2	F2	-	82-10/02/90	10/25/90	E2 - 10/26/90	35-40	51-56	25-26	31-32
I	I	PATH	0	D2	F2	-	85-10/08/90	10/31/90	E3 <sup>c</sup> - 11/01/90	67-72	91-96	13-14	19-20
I	I	PATH	0	D2	F2	-	86-10/09/90	11/01/90	E4 - 11/02/90	115-120	139-144	37-38	43-44
IR	IR	LAV	0	D2	F2	+	81-10/01/90	10/24/90	R1 <sup>c</sup> - 11/08/90	9-10	25-26	3	9
IR	IR	LAV	0	D2	F2	+	82-10/02/90	10/25/90	R2 - 11/09/90	41-42	57-58	27	33
IR	IR	LAV	0	D2	F2	+	85-10/08/90	10/31/90	R3 <sup>c</sup> - 11/15/90	73-74	97-98	15	21
IR	IR	LAV	0	D2	F2	+	86-10/09/90	11/01/90	R4 - 11/16/90	121-122	145-146	39	45
IR	IR	PF	0	D2	F2	+	85-10/08/90	10/31/90	R1P <sup>c</sup> - 11/15/90	83-84	107-108	17	23
IR	IR	PF	0	D2	F2	+	85-10/08/90	11/01/90	R2P - 11/16/90	87-88	111-112	18	24
IR	IR	PF	0	D2	F2	+	86-10/09/90	11/02/90	R3P - 11/17/90	131-132	155-156	41	47
IR	IR	PF	0	D2	F2	+	86-10/09/90	11/03/90	R4P <sup>d</sup> - 11/18/90	135-136	159-160	42	48
IR	IR	PATH	0	D2	F2	+	81-10/01/90	10/24/90	R1 <sup>c</sup> - 11/08/90	11-16	27-32	3-4	9-10
IR	IR	PATH	0	D2	F2	+	82-10/02/90	10/25/90	R2 - 11/09/90	43-48	59-64	27-28	33-34
IR	IR	PATH	0	D2	F2	+	85-10/08/90	10/31/90	R3 <sup>c</sup> - 11/15/90	75-80	99-104	15-16	21-22
IR	IR	PATH	0	D2	F2	+	86-10/09/90	11/02/90	R4 - 11/16/90	123-128	147-152	39-40	45-46
II	II	LAV	C1	D1	F1	-	83-10/03/90	10/24/90	E1 <sup>c</sup> - 10/25/90	361-363	-	73	-
II	II	PF	C1	D1	F1	-	87-10/10/90	10/31/90	E1P <sup>c</sup> - 11/01/90	385-387	-	121	-
II	II	PATH	C1	D1	F1	-	83-10/03/90	10/24/90	E1 <sup>c</sup> - 10/25/90	364-372	-	73-75	-
II	II	LAV	C1	D1	F1	+	83-10/03/90	10/24/90	R1 <sup>c</sup> - 11/08/90	-	373-375	-	76
II	II	PF	C1	D1	F1	+	87-10/10/90	10/31/90	R1P <sup>c</sup> - 11/15/90	-	403-405	-	127
II	II	PATH	C1	D1	F1	+	83-10/03/90	10/24/90	R1 <sup>c</sup> - 11/08/90	-	376-384	-	76-78

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<sup>a</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles. R = REC (assayed after a 14 day recovery period).

<sup>b</sup> S = Exposure Start Days 1 to 8 are staggered over a two-week period; E = Assay days 1 to 4 for the EXP (within 24 hr of the last exposure) endpoint timing; R = Assay days 1 to 4 for the REC (after a 14 day recovery period) endpoint timing; P = Pulmonary function EXP and REC assays are scheduled to occur on four consecutive days each.

<sup>c</sup> For F2 and F1 groups, the fourth week of exposures will be conducted Sunday through Wednesday or Tuesday and Wednesday, respectively, to accommodate endpoint measurements on Thursday.

<sup>d</sup> For F2 and F1 PF groups, the fourth week of exposures will be conducted Wednesday through Saturday or Friday and Saturday, respectively, to accommodate endpoint measurements on Sunday.

**Attachment B. Summary of Animal Numbers and Cage Locations of Rats used  
for the Various Exposure Conditions and Various Endpoints.  
(Continued)**

Expo. Class	Expt Code	Expo. Group	Expo. Conc.	Expo. hr/day	Expo. freq.	Recovery	Exposure		Experiment Date <sup>b</sup>	Animal Numbers		Case Number(s)	
							Start <sup>b</sup>	End		Male <sup>a</sup>	Female <sup>a</sup>	Male <sup>a</sup>	Female <sup>a</sup>
III	LAV	C2	D1	F1	-	-	58-10/11/90	11/01/90	E4	-	469-471	-	141
III	PF	C2	D1	F1	-	-	58-10/11/90	11/03/90	E4P <sup>d</sup>	-	463-465	-	139
III	PATM	C2	D1	F1	-	-	58-10/11/90	11/01/90	E4	-	472-480	-	141-143
IIIR	LAV	C2	D1	F1	+	+	58-10/11/90	11/01/90	R4	451-453	-	135	-
IIIR	PF	C2	D1	F1	+	+	58-10/11/90	11/03/90	R4P <sup>d</sup>	445-447	-	133	-
IIIR	PATM	C2	D1	F1	+	+	58-10/11/90	11/01/90	R4	454-462	-	135-137	-
IV	LAV	C1	D1	F2	-	-	52-10/02/90	10/25/90	E2	-	281-283	-	67
IV	PF	C1	D1	F2	-	-	55-10/08/90	11/01/90	E2P	-	246-248	-	106
IV	PATM	C1	D1	F2	-	-	52-10/02/90	10/25/90	E2	-	284-292	-	67-69
IVR	LAV	C1	D1	F2	+	+	52-10/02/90	10/25/90	R2	261-263	-	61	-
IVR	PF	C1	D1	F2	+	+	55-10/08/90	11/01/90	R2P	216-218	-	100	-
IVR	PATM	C1	D1	F2	+	+	52-10/02/90	10/25/90	R2	264-272	-	61-63	-
V	LAV	C2	D1	F2	-	-	55-10/08/90	10/31/90	E3 <sup>c</sup>	204-206	-	97	-
V	PF	C2	D1	F2	-	-	56-10/09/90	11/02/90	E3P	307-309	-	113	-
V	PATM	C2	D1	F2	-	-	55-10/08/90	10/31/90	E3 <sup>c</sup>	207-215	-	97-99	-
VR	LAV	C2	D1	F2	+	+	55-10/08/90	10/31/90	R3 <sup>c</sup>	-	234-236	-	103
VR	PF	C2	D1	F2	+	+	56-10/09/90	11/02/90	R3P	-	310-312	-	114
VR	PATM	C2	D1	F2	+	+	55-10/08/90	10/31/90	R3 <sup>c</sup>	-	237-245	-	103-105
VI	LAV	C1	D2	F1	-	-	57-10/10/90	10/31/90	E3 <sup>c</sup>	-	409-411	-	129
VI	PF	C1	D2	F1	-	-	58-10/11/90	11/02/90	E3P	-	466-468	-	140
VI	PATM	C1	D2	F1	-	-	57-10/10/90	10/31/90	E3 <sup>c</sup>	-	412-420	-	129-131
VIR	LAV	C1	D2	F1	+	+	57-10/10/90	10/31/90	R3 <sup>c</sup>	391-393	-	123	-
VIR	PF	C1	D2	F1	+	+	58-10/11/90	11/02/90	R3P	448-450	-	135	-
VIR	PATM	C1	D2	F1	+	+	57-10/10/90	10/31/90	R3 <sup>c</sup>	394-402	-	123-125	-
VII	LAV	C2	D2	F1	-	-	54-10/04/90	10/25/90	E2	421-423	-	79	-
VII	PF	C2	D2	F1	-	-	57-10/10/90	11/01/90	E2P	388-390	-	122	-
VII	PATM	C2	D2	F1	-	-	54-10/04/90	10/25/90	E2	424-432	-	79-81	-
VIIIR	LAV	C2	D2	F1	+	+	54-10/04/90	10/25/90	R2	-	433-435	-	82
VIIIR	PF	C2	D2	F1	+	+	57-10/10/90	11/01/90	R2P	-	404-408	-	128
VIIIR	PATM	C2	D2	F1	+	+	54-10/04/90	10/25/90	R2	-	436-444	-	82-84
VIIIR	LAV	C1	D2	F2	-	-	56-10/09/90	11/01/90	E4	349-351	-	88	-
VIIIR	PF	C1	D2	F2	-	-	56-10/09/90	11/03/90	E4P <sup>d</sup>	301-303	-	111	-
VIIIR	PATM	C1	D2	F2	-	-	56-10/09/90	11/01/90	E4	352-360	-	88-90	-
VIIIR	LAV	C1	D2	F2	+	+	56-10/09/90	11/01/90	R4	-	337-339	-	85
VIIIR	PF	C1	D2	F2	+	+	56-10/09/90	11/03/90	R4P <sup>d</sup>	-	304-306	-	112
VIIIR	PATM	C1	D2	F2	+	+	56-10/09/90	11/01/90	R4	-	340-348	-	85-87

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- <sup>a</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles. R = REC (assayed after a 14 day recovery period).
- <sup>b</sup> s = Exposure Start Days 1 to 8 are staggered over a two-week period; E = Assay days 1 to 4 for the EXP (within 24 hr of the last exposure) endpoint timing; R = Assay days 1 to 4 for the REC (after a 14 day recovery period) endpoint timing; P = Pulmonary function EXP and REC assays are scheduled to occur on four consecutive days each.
- <sup>c</sup> For F2 and F1 groups, the fourth week of exposures will be conducted Sunday through Wednesday or Tuesday and Wednesday, respectively, to accommodate endpoint measurements on Thursday.
- <sup>d</sup> For F2 and F1 Pf groups, the fourth week of exposures will be conducted Wednesday through Saturday or Friday and Saturday, respectively, to accommodate endpoint measurements on Sunday.

**Attachment B. Summary of Animal Numbers and Cage Locations of Rats Used  
for the Various Exposure Conditions and Various Endpoints.  
(Continued)**

Expo. Class	Exptl Group	Expo. Conc.	Expo. hr/day	Expo. Frag.	Expo. Recovery	Exposure		Experiment Date <sup>b</sup>	Animal Numbers		Cage Number(s)	
						Start <sup>b</sup>	End		Males	Females	Males	Females
IX	LAV	C2	D2			81-10/01/90	10/24/90	E1 <sup>c</sup> - 10/25/90	-	181-183	-	55
IX	PF	C2	D2			85-10/08/90	10/31/90	E1P <sup>c</sup> - 11/01/90	-	231-233	-	60
IX	PATN	C2	D2			81-10/01/90	10/24/90	E1 <sup>c</sup> - 10/25/90	-	184-192	-	55-57
IXR	LAV	C2	D2			81-10/01/90	10/24/90	R1 <sup>c</sup> - 11/08/90	161-163	-	49	-
IXR	PF	C2	D2			85-10/08/90	10/31/90	R1P <sup>c</sup> - 11/13/90	201-203	-	54	-
IXR	PATN	C2	D2			81-10/01/90	10/24/90	R1 <sup>c</sup> - 11/08/90	164-172	-	49-51	-
X	LAV	P	D2			81-10/01/90	10/24/90	E1 <sup>c</sup> - 10/25/90	173-175	193-193	52	58
X	LAV	P	D2			82-10/02/90	10/25/90	E2 - 10/26/90	273-273	293-293	64	70
X	LAV	P	D2			85-10/08/90	10/31/90	E3 <sup>c</sup> - 11/01/90	219-219	249-249	101	107
X	LAV	P	D2			86-10/09/90	11/01/90	E4 - 11/02/90	317-317	329-329	117	119
X	PF	P	D2			85-10/08/90	10/31/90	E1P <sup>c</sup> - 11/01/90	227-227	257-257	109	109
X	PF	P	D2			85-10/08/90	11/01/90	E2P - 11/02/90	229-229	259-259	110	110
X	PF	P	D2			86-10/09/90	11/02/90	E3P - 11/03/90	313-313	325-325	115	115
X	PF	P	D2			86-10/09/90	11/03/90	E4P <sup>d</sup> - 11/04/90	315-315	327-327	116	116
X	PATN	P	D2			81-10/01/90	10/24/90	E1 <sup>c</sup> - 10/25/90	174-176	194-196	52	58
X	PATN	P	D2			82-10/02/90	10/25/90	E2 - 10/26/90	274-276	294-296	64	70
X	PATN	P	D2			85-10/08/90	10/31/90	E3 <sup>c</sup> - 11/01/90	220-222	250-252	101	107
X	PATN	P	D2			86-10/09/90	11/01/90	E4 - 11/02/90	316-320	330-332	117	119
XR	LAV	P	D2			81-10/01/90	10/24/90	R1 <sup>c</sup> - 11/08/90	177-177	197-197	53	59
XR	LAV	P	D2			82-10/02/90	10/25/90	R2 - 11/09/90	277-277	297-297	65	71
XR	LAV	P	D2			85-10/08/90	10/31/90	R3 <sup>c</sup> - 11/15/90	223-223	253-253	102	108
XR	LAV	P	D2			86-10/09/90	11/01/90	R4 - 11/16/90	321-321	333-333	118	120
XR	PF	P	D2			85-10/08/90	10/31/90	R1P <sup>c</sup> - 11/15/90	228-228	258-258	109	109
XR	PF	P	D2			85-10/08/90	11/01/90	R2P - 11/16/90	230-230	260-260	110	110
XR	PF	P	D2			86-10/09/90	11/02/90	R3P - 11/17/90	314-314	326-326	115	115
XR	PF	P	D2			86-10/09/90	11/03/90	R4P <sup>d</sup> - 11/18/90	316-316	328-328	116	116
XR	PATN	P	D2			81-10/01/90	10/24/90	R1 <sup>c</sup> - 11/08/90	178-180	198-200	53	59
XR	PATN	P	D2			82-10/02/90	10/25/90	R2 - 11/09/90	278-280	298-300	65	71
XR	PATN	P	D2			85-10/08/90	10/31/90	R3 <sup>c</sup> - 11/15/90	224-226	254-256	102	108
XR	PATN	P	D2			86-10/09/90	11/01/90	R4 - 11/16/90	322-324	334-336	118	120

<sup>a</sup> Exposure Classes II - IX receive aerosols of the test article. Exposure Class I is filtered air control; Exposure Class X receives aerosols of positive control particles. R = REC (assayed after a 14 day recovery period).

<sup>b</sup> S = Exposure Start Days 1 to 8 are staggered over a two-week period; E = Assay days 1 to 4 for the EXP (within 24 hr of the last exposure) endpoint timing; R = Assay days 1 to 4 for the REC (after a 14 day recovery period) endpoint timing; P = Pulmonary function EXP and REC assays are scheduled to occur on four consecutive days each.

<sup>c</sup> For F2 and F1 groups, the fourth week of exposures will be conducted Sunday through Wednesday or Tuesday and Wednesday, respectively, to accommodate endpoint measurements on Thursday.

<sup>d</sup> For F2 and F1 PF groups, the fourth week of exposures will be conducted Wednesday through Saturday or Friday and Saturday, respectively, to accommodate endpoint measurements on Sunday.

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PROTOCOL AMENDMENT 2

Study Title: FOUR-WEEK REPEATED DOSE INHALATION TOXICITY STUDY WITH  
AEROSOLS OF A SOLID PARTICULATE TEST MATERIAL IN MALE  
AND FEMALE F344/N RATS TO EVALUATE THE EFFECTS OF  
EXPOSURE CONCENTRATION, DURATION, FREQUENCY, AND  
RECOVERY TIME ON VARIOUS BIOLOGICAL ENDPOINTS

Effective Date: October 1, 1990

Amendment

Change: Attachment A (Housing Position Map) is revised to move the  
cages housing animals exposed to the positive control  
particle to Rack 7 when their exposure starts.

Reason: Decision was made to isolate the positive control animals  
from the test particle-exposed animals after their  
exposure.

Approval:

Study Director:

Jeannie Bradof  
Jeannie Bradof

Date: 10/1/90

Principal  
Investigator:

Catherine Aranyi  
Catherine Aranyi

Date: 10/1/90

Quality  
Assurance  
Manager:

Ron Boyne by K.P.  
Ronald Boyne

Date: 10/1/90

Sponsor (COTR):

Jack P. Dacre  
Dr. Jack Dacre

Date: 10/17/90

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# ATTACHMENT A. (REVISED) HOUSING POSITION MAP

BACK 1

Comp. #

Comp. #

1	1	2	3	4
2	5	6	7	8
3	9	10	11	12
4	13	14	15	16
5	X	X	X	X
6	X	X	X	X

7	17	18	19	20
8	21	22	23	24
9	25	26	27	28
10	29	30	31	32
11	X	X	X	X
12	X	X	X	X

BACK 2

Comp. #

Comp. #

25	33	34	35	36
26	37	38	39	40
27	41	42	43	44
28	45	46	47	48
29	X	X	X	X
30	X	X	X	X

31	49	50	51	52
32	53	54	55	56
33	57	58	59	60
34	61	62	63	64
35	X	X	X	X
36	X	X	X	X

13	65	66	67	68
14	69	70	71	72
15	73	74	75	76
16	77	78	79	80
17	81	82	83	84
18	85	86	87	88

19	89	90	91	92
20	93	94	95	96
21	97	98	99	100
22	101	102	103	104
23	105	106	107	108
24	109	110	111	112

37	113	114	115	116
38	117	118	119	120
39	121	122	123	124
40	125	126	127	128
41	129	130	131	132
42	133	134	135	136

43	137	138	139	140
44	141	142	143	144
45	145	146	147	148
46	149	150	151	152
47	153	154	155	156
48	157	158	159	160

X = Empty Compartment

# ATTACHMENT A. (REVISED) HOUSING POSITION MAP (cont'd)

RACK 3

Cage #

Cage #

49	161	162	163	164
50	165	166	167	168
51	169	170	171	172
52	173	174	175	176
53	177	178	179	180
54*	201	202	203	X

55	181	182	183	184
56	185	186	187	188
57	189	190	191	192
58	193	194	195	196
59	197	198	199	200
60*	231	232	233	X

RACK 4

Cage #

Cage #

73	361	362	363	364
74	365	366	367	368
75	369	370	371	372
76	373	374	375	376
77	377	378	379	380
78	381	382	383	384

79	421	422	423	424
80	425	426	427	428
81	429	430	431	432
82	433	434	435	436
83	437	438	439	440
84	441	442	443	444

61	261	262	263	264
62	265	266	267	268
63	269	270	271	272
64	273	274	275	276
65	277	278	279	280
66	X	X	X	X

67	281	282	283	284
68	285	286	287	288
69	289	290	291	292
70	293	294	295	296
71	297	298	299	300
72	X	X	X	X

85*	337	338	339	340
86*	341	342	343	344
87*	345	346	347	348
88*	349	350	351	352
89*	353	354	355	356
90*	357	358	359	360

91	X	X	X	X
92	X	X	X	X
93	X	X	X	X
94	X	X	X	X
95	X	X	X	X
96	X	X	X	X

\* Cages #54 and #60 will be housed in cage positions #6 and # 12 (in the control animal room) until their exposure start.  
 \* Cages #52, 53, 58, 59, 64, 65, 70, and 71 will be moved to Rack 7 once their exposure to the positive control particle starts.

\* Cages #85 to 90 will be housed in cage positions #9, 11, 29-30, and 35-36 (in the control animal room) until their exposure start.

X = Empty Compartment

# ATTACHMENT A. (REVISED) HOUSING POSITION MAP (cont'd)

RACK 5

Cage #

Cage #

97	204	205	206	207	103	234	235	236	237
98	208	209	210	211	104	238	239	240	241
99	212	213	214	215	105	242	243	244	245
100	216	217	218	X	106	246	247	248	X
101	219	220	221	222	107	249	250	251	252
102	223	224	225	226	108	253	254	255	256

RACK 6

Cage #

Cage #

121	305	306	307	X	127	403	404	405	X
122	308	309	390	X	128	406	407	408	X
123	391	392	393	394	129	409	410	411	412
124	395	396	397	398	130	413	414	415	416
125	399	400	401	402	131	417	418	419	420
126	X	X	X	X	132	X	X	X	X

109	227	228	229	230	231	232	233	234	235	236
110	229	230	231	232	233	234	235	236	237	238
111	301	302	303	X	304	305	306	X	307	308
112	304	305	306	X	307	308	309	X	310	311
113	307	308	309	X	310	311	312	X	313	314
114	310	311	312	X	313	314	315	316	317	318

115	313	314	315	316	317	318	319	320	321	322
116	315	316	317	318	319	320	321	322	323	324
117	317	318	319	320	321	322	323	324	325	326
118	321	322	323	324	325	326	327	328	329	330
119	329	330	331	332	333	334	335	336	337	338
120	333	334	335	336	337	338	339	340	341	342

133	445	446	447	X	139	463	464	465	X
134	448	449	450	X	140	466	467	468	X
135	451	452	453	454	141	469	470	471	472
136	455	456	457	458	142	473	474	475	476
137	459	460	461	462	143	477	478	479	480
138	X	X	X	X	144	X	X	X	X

• Cages #101, 102, 108 - 110, and 115 - 120 will be moved to Rack 7 once their exposure to the positive control particle starts.

X = Empty Compartment



ATTACHMENT A. (REVISED) HOUSING POSITION MAP (cont'd)

RACK 7

52	173	174	175	176	101	219	220	221	222
53	177	178	179	180	102	223	224	225	226
58	193	194	195	196	108	233	234	235	236
59	197	198	199	200	109	227	228	237	238
	X	X	X	X	110	229	230	231	232
	X	X	X	X		X	X	X	X

64	273	274	275	276	115	313	314	325	326
65	277	278	279	280	116	315	316	327	328
70	293	294	295	296	117	317	318	319	320
71	297	298	299	300	118	321	322	323	324
	X	X	X	X	119	329	330	331	332
	X	X	X	X	120	333	334	335	336

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X = Empty Compartment

PROTOCOL AMENDMENT 3

Study Title: **FOUR-WEEK REPEATED DOSE INHALATION TOXICITY STUDY WITH AEROSOLS OF A SOLID PARTICULATE TEST MATERIAL IN MALE AND FEMALE F344/N RATS TO EVALUATE THE EFFECTS OF EXPOSURE CONCENTRATION, DURATION, FREQUENCY, AND RECOVERY TIME ON VARIOUS BIOLOGICAL ENDPOINTS**

Effective Date: October 11, 1990

In the following listing of changes, additions or changes are indicated with **bold type and underlined**. To provide the context for the changes and simplify presentation, the section and subsection numbers are given in which the changes are made and the relevant paragraph(s) adjacent to the changes are included.

Amendment

Change: Section XII.1.2 is changed as follows.

XII.1.2 Clinical Observations:

All animals will be observed twice **daily on weekdays** at least six hours apart (prior to 10:00 AM and after 2:00 PM) **(once daily on the weekends)** for moribundity and mortality (SOP: NTP-420R3). Each animal will be formally examined twice weekly, once in the morning (Mondays for Exposure Start Groups S1, S3, S5, and S7 and Tuesdays for Exposure Start Groups S2, S4, S6, and S8) and once in the afternoon (Thursdays for Exposure Start Groups S1, S3, S5, and S7 and Fridays for Exposure Start Groups S2, S4, S6, and S8), for clinical signs of pharmacologic and toxicologic effects of the exposure (SOP: GT-401R1). On exposure days the first mortality/ moribundity observation will coincide with food removal and chamber loading operations.

The study toxicologist, or veterinarian, will visit the laboratory at least once a week to confirm, correct or expand the clinical observations. Any **PATH-designated** animal whose condition suggests that it may not survive until the next observation based upon established criteria (SOP: NTP-409R4) will be euthanized and necropsied immediately, with the tissues retained in formalin. Gross lesions found at necropsy will be documented and the affected tissues processed for histopathological examination. **Moribund animals designated for PF or LAV will be euthanized and the carcasses disposed of without necropsy or tissue collection.**

Reasons: Paragraph 1: Statement needed clarification. Paragraph 2: Clarification that, among moribund animals, necropsies and tissue collection will occur for PATH-designated animals only.

Change: The first and last paragraphs of Section XII.2.2 are changed as indicated.

XII.2.2 Necropsy:

All scheduled necropsies will be performed in the presence of and under the supervision of the IITRI-PAI pathologist. All unscheduled necropsies (moribund sacrifice and spontaneous deaths of PATH-designated animals) will be supervised by the pathologist to the maximum extent possible, and will be performed as soon after death as possible. Animals for unscheduled necropsies will not be frozen, and every attempt will be made to refrigerate animals for no longer than eight hours prior to the necropsy. Animals will be euthanized with CO<sub>2</sub> according to SOP: NTP-421R1.

Rats designated for scheduled necropsy and histopathologic examination will be anesthetized with carbon dioxide and exsanguinated from the abdominal aorta, either within 24 hr following the last exposure or after a two-week recovery period following the exposures. All scheduled necropsies will be initiated promptly after an animal is killed and will be performed on all PATH-designated animals.

Reason: Clarification that only PATH-designated animals will be necropsied.

Approval:

Study Director: Jeannie Bradof Date: 10/11/90  
Jeannie Bradof

Principal Investigator: Catherine Aranyi Date: 10/11/90  
Catherine Aranyi

Quality Assurance Manager: Ronald Boyne Date: 10/11/90  
Ronald Boyne

Sponsor (COTR): Jack P. Dacre Date: 10/18/90  
Dr. Jack Dacre

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PROTOCOL DEVIATIONS 1, 2 and 3

Study Title: FOUR-WEEK REPEATED DOSE INHALATION TOXICITY STUDY WITH AEROSOLS OF A SOLID PARTICULATE TEST MATERIAL IN MALE AND FEMALE F344/N RATS TO EVALUATE THE EFFECTS OF EXPOSURE CONCENTRATION, DURATION, FREQUENCY, AND RECOVERY TIME ON VARIOUS BIOLOGICAL ENDPOINTS

Date: March 12, 1991

Protocol Deviation

1. Due to malfunction of one of the PCAMs on October 18, 1990, one PCAM had to be shared between Chambers 1 (1 hr, 100 mg/m<sup>3</sup> graphite) and 2 (1 hr, 200 mg/m<sup>3</sup> graphite) for the remaining exposure days in the study. The PCAM probe was moved back and forth between the two chambers as soon as a stable reading was obtained on the LED. The printouts of the 5 minute averages obtained were therefore not meaningful and could not be used to generate mean PC values to correlate with the gravimetric samples for those chambers.
2. Due to malfunction of that portion of the pulmonary function software, the end expiratory volume (FRC) could not be measured.
3. In order to correlate more closely with the food consumption measurements, the body weights were calculated as weight gain per interval instead of as change relative to the body weight measurement taken prior to the first exposure.

Study Director:

Jeannie Bradof  
Jeannie Bradof

Date: 3/12/91

CONTRACT NO.: DAMD17-89-C-9043  
IITRI PROJECT NO.: L06234

INHALATION TOXICITY OF SINGLE MATERIALS AND MIXTURES:  
PHASE II - FOUR-WEEK INHALATION TOXICITY STUDY  
OF A SOLID PARTICULATE AEROSOL IN F344/N RATS

## PART TWO

# STATISTICAL OVERVIEW OF THE RESULTS

Prepared for the Life Sciences Research Department  
of IIT Research Institute

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## EXECUTIVE SUMMARY TO PART TWO

Multivariate analysis of variance models (Bock, 1975) were used to analyze each set of variables (*i.e.*, hematology, pulmonary lavage, hematology differentials, clinical chemistry, body weight gain, relative lung weight, food consumption, and pulmonary function). Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: sex (male or female), period (post-exposure or post-recovery), concentration (control, 100  $mg/m^3$ , 200  $mg/m^3$ , or positive control at 200  $mg/m^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made. Multivariate analysis of variance models (Bock, 1975) were used to analyze the body weight gains, and food consumption.

The results of the statistical computations revealed a remarkably striking absence of significant main effects of any of these factors, suggesting that differences in levels of concentration, frequency, and duration are unrelated to outcome. A notable exception, however, were the pulmonary lavage parameters. Here, several measurements were effected by all three factors, either significantly increased or decreased. In addition, lung weights and several hematology differentials (WBC, NEUT, MONO, and EOS) were significantly increased at the high duration relative to the low duration, but were seemingly uninfluenced by concentration and frequency.

In terms of significant interactions, most predominant were interactions involving concentration and duration. These interactions generally involved either decreased or increased response levels in animals exposed to the low concentration and high duration combination. Similarly, the few concentration by frequency interactions involved either decreased or increased response levels in animals exposed to the low concentration and high frequency combination. The only measurements that appeared to be either increased or decreased in the high concentration, high frequency or high duration groups were PHAGO, PROTEIN, and BW GAIN. In general, however, only the pulmonary lavage variables appeared to be sensitive to changes in concentration, duration, or frequency.

When compared to controls, post-exposure sacrifice animals exhibited relatively few exposure related effects. Significant decreases in ALP, BW GAIN, MCV, PLT, MONO, FVC, TLC, and VC were observed, although the latter three pulmonary function measures were only significant following covariate adjustment for body weight. Significant increases in TRIG, NEUT, PROTEIN, and FOB were observed and again, the pulmonary function measure FOB, was only significant following covariate adjustment for body weight.

For post-recovery sacrifice animals, significant decreases in ALT, TP, CA, FC, MONO, RL, FVC, DLCO, and VC were observed relative to control animals, although the latter three pulmonary function measures were only significant following covariate adjustment for body weight. Significant increases in CHOL, CREA, TRIG, BW GAIN, REL LUNG WT, PLT, WBC, NEUT, MONO, TOTVCELL, TOTCELL, and NEUT were observed. Interestingly, in males, TP and CA were decreased, but they were increased in females. In addition, the hematology differential increases in WBC, NEUT, and MONO were restricted to low concentration males. Decreases in pulmonary function measures FVC, DLCO, and VC were restricted to low concentration animals, however decreases in RL levels were seen in both low and high concentration animals.

In summary, this study has shown that with the exception of pulmonary lavage measures, concentration, duration, and frequency had little if any effect on outcome. Relative to controls,



consistent exposure related decreases were observed for ALP, BW, MCV, PLT, MONO, FVC, TLC, and VC, and exposure related increases for NEUT (hematology differential), NEUT (pulmonary lavage), PROTEIN, and FOB, although the effects for the pulmonary function measures were only significant following covariate adjustment for body weight. Following the recovery period consistent decreases in RL, FC, and ALT, and increases in LUNG, TOTVCELL, TOTCELL, and NEUT were observed.

## References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill. 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*. 2nd edition. New York: McGraw-Hill. 1971.

## 1 Introduction

The purpose of Part Two of this report is to provide an overview of the voluminous statistical effort that has been undertaken in the analysis of these data, presented in the Part One Technical Report. The report begins by presenting an overview of the statistical methods used in the analysis of the various sets of outcome measures (*i.e.*, hematology, clinical chemistry, etc.). Next, a global summary of effects of concentration, frequency, and duration, and their interactions are described for all measured outcome variables. Next, a summary of significant differences from control is presented, which identifies treatment related effects for males and females, at low and high concentrations, at the post-exposure and post-recovery sacrifice. Finally, a summary of the results for each set of outcome measures is presented. This summary goes beyond the previous presentation by including effects that may depend on combinations of all five experimental factors (*i.e.*, concentration, duration, frequency, sex and period).

## 2 Statistical Methodology

Multivariate analysis of variance models (Bock, 1975) were used to analyze each set of variables (*i.e.*, hematology, pulmonary lavage, hematology differentials, clinical chemistry, body weight gain, relative lung weight, food consumption, and pulmonary function. Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: sex (male or female), period (post-exposure or post-recovery), concentration (control, 100  $mg/m^3$ , 200  $mg/m^3$ , or positive control at 200  $mg/m^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the

exception that only the graphite-exposed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the graphite exposed groups were examined using a multivariate analysis of variance model which included all five factors and all two-way interactions. The second set of analyses concentrated on examining the differences between the exposed and control animals. These analyses were performed separately in each of the four subsamples, defined by period and sex, in order to examine group differences while controlling for the effects of period and sex. First, a one-factor multivariate analysis of variance was performed on the measures in a particular category (*e.g.*, the eight hematology measures), with concentration as the grouping factor, augmented by simple contrasts which allowed a statistical comparison to be made between each group ( $100\text{ mg/m}^3$ ,  $200\text{ mg/m}^3$ , and positive control at  $200\text{ mg/m}^3$ ) and the control group. In order to examine the consistency of any group differences between the levels of frequency and duration, a second multivariate analysis of variance was performed. In this second analysis, the low and high concentration animals were further grouped depending on their duration and frequency of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the graphite exposed and control groups was consistent across the duration and frequency subgroups.

Multivariate analysis of variance models (Bock, 1975) were used to analyze the body weight gains. For the analysis of the post-exposure period, weekly body weight gain values for weeks 1 through 3 were obtained by summing, for each week, the two weekly body weight gain determinations. A multivariate analysis of variance for repeated measures was then performed, utilizing orthogonal polynomial contrasts, to examine for constant and linear trend effects over the first three weeks of the exposure period. While animals were assessed twice during week four in the exposure period, many animals were missing one of these assessments, and so the analysis was performed

simply on the first three weeks. A multivariate analysis of variance was also performed using the two recovery assessments as dependent variables in order to examine any potential changes in body weight gains during the recovery period.

Multivariate analysis of variance models for repeated measures were also used to analyze food consumption. In these analyses, polynomial contrasts were utilized to test for constant effects across time as well as for linear trends across time. Although two food consumption assessments were obtained per week, these two values were averaged to yield an average food consumption value for each of the five weeks. Food consumption was only measured in recovery animals.

A detailed statistical report on all of the statistical tests performed, as well as relevant summary statistics is presented in sections A-II of the Appendix. A summary of these results is now presented.

### **3 Results for Graphite Exposed Animals**

A summary of all significant main effects and interactions involving concentration, frequency, and duration is presented in Table 1. Inspection of Table 1 reveals a remarkably striking absence of significant main effects of any of these factors, suggesting that differences in levels of concentration, frequency, and duration are unrelated to outcome. A notable exception, however, were the pulmonary lavage parameters. Here, several measurements were effected by all three factors, either significantly increased or decreased. In addition, lung weights and several hematology differentials (WBC, NEUT, MONO, and EOS) were significantly increased at the high duration relative to the low duration, but were seemingly uninfluenced by concentration and frequency.

In terms of significant interactions, most predominant were interactions involving concentration and duration. These interactions generally involved either decreased or increased response levels in animals exposed to the low concentration and high duration combination. Similarly, the few concentration by frequency interactions involved either decreased or increased response levels in

animals exposed to the low concentration and high frequency combination. The only measurements that appeared to be either increased or decreased in the high concentration, high frequency or high duration groups were PHAGO, PROTEIN, and BW GAIN. In general, however, only the pulmonary lavage variables appeared to be sensitive to changes in concentration, duration, or frequency.

#### 4 Comparisons to Controls

A summary of comparisons of exposed versus control animals is presented in Table 2 for post-exposure sacrifice animals, and Table 3 for post-recovery sacrifice animals. Tables 2 and 3 summarize the significant increases and decreases relative to controls, in terms of sex and concentration. Inspection of Table 2, for post-exposure sacrifice animals, reveals relatively few treatment related effects. Significant decreases in ALP, BW GAIN, MCV, PLT, MONO, FVC, TLC, and VC were observed, although the latter three pulmonary function measures were only significant following covariate adjustment for body weight. Significant increases in TRIG, NEUT, PROTEIN, and FOB were observed and again, the pulmonary function measure FOB, was only significant following covariate adjustment for body weight.

Inspection of Table 3, for post-recovery sacrifice animals, reveals some additional treatment related effects. Significant decreases in ALT, TP, CA, FC, MONO, RL, FVC, DLCO, and VC were observed, although the latter three pulmonary function measures were only significant following covariate adjustment for body weight. Significant increases in CHOL, CREA, TRIG, BW GAIN, REL LUNG WT, PLT, WBC, NEUT, MONO, TOTVCELL, TOTCELL, and NEUT were observed. Interestingly, in males, TP and CA were decreased, but they were increased in females. In addition, the hematology differential increases in WBC, NEUT, and MONO were restricted to low concentration males. Decreases in pulmonary function measures FVC, DLCO, and VC were restricted to low concentration animals, however decreases in RL levels were seen in both low and

high concentration animals.

## 5 More Detailed Summary of Significant Results

In each of the following subsections, a summary of the significant results is presented. This summary reflects all of the analyses that are reported in the Statistical Appendix, and not just the overall summary presented in Tables 1, 2 and 3.

### 5.1 Hematology

In general, duration and frequency did not play a role in the effect of exposure to the compound. Male animals did not appear to exhibit any major exposure related effects at the post-exposure sacrifice, although WBC and PLT levels were increased relative to controls following the recovery period. Female animals exhibited consistent decreases in MCV and PLT levels at the treatment sacrifice for both concentrations, but PLT values were increased relative to controls following the recovery period. When the differential effects of duration and frequency were examined, low dose male animals exposed for 4 hours 4 times per week did exhibit increases in RBC, HGB, and HCT, but this effect was not reproduced at the high dose or following the recovery period. Following the recovery period several significant increases (RBC, HCT, MCHC, and PLT) were observed for female animals at various concentrations, durations and frequencies. The most consistent effect was for PLT. In addition, high dose female animals did exhibit significant decreases in HCT, MCH and MCHC following the recovery period, but these differences were not consistent for any particular frequency or duration.

In summary, exposure to this compound produced consistent decreases in MCV and PLT for female animals regardless of concentration, duration, and frequency. Male animals were relatively unaffected. Following the recovery period the picture was somewhat more complex with both male

and female animals exhibiting increased PLT levels, although a scatter of significant increases and decreases for several different outcome measures were observed when the data were broken down by duration and frequency. However, no consistent duration or frequency related effects were observed.

## **5.2 Pulmonary Lavage**

Overall, low concentration post-exposure animals exhibited significantly increased NEUT relative to controls. At the high concentration, both NEUT and PROTEIN were elevated and significant decreases were observed for MONO. These effects appeared to be most pronounced for the 4 hour per day duration and 4 time per week exposure frequency. In fact, in high concentration animals virtually identical significant differences were observed for 4 hour per week 4 time per week animals as for positive controls. For the low duration and low frequency conditions, both high and low dose animals appeared to normalize following recovery, but this was not true for positive controls or high duration and/or high frequency animals.

## **5.3 Hematology Differentials**

In general, duration and frequency did not play a major role in the effect of exposure to the compound. Both male and female concentration dose animals exhibited significantly increased NEUT levels relative to controls, but this effect was not present following the recovery period.

## **5.4 Clinical Chemistry**

Overall, the main effects of concentration, duration, and frequency were not significant, suggesting that when averaged over sex and period, little difference in the levels of these factors (*i.e.*, low or high, 1 or 4 hrs. or 2 or 4 times per week) was observed. Numerous interactions among these factors, however, revealed that the results are not quite so easy to interpret. For example, little if anything was significant for male post-exposure sacrifice animals, whereas females exhibited consistently

increased TRIG levels and decreased ALP and PHOS levels. When broken down by frequency and duration, it appeared that females again had consistently elevated TRIG levels regardless of concentration, frequency, or duration, and both male and females exhibited decreased SDH levels for both concentrations and various combinations of frequency and duration. In addition, reasonably consistent decreases in ALP and PHOS were also observed. Following the recovery period the effects on TRIG remained unchanged, but SDH, ALP, and PHOS appeared to return to normal levels. However, after the recovery period, several other consistent increases (TP, CA) and decreases (ALT, ALBG, and TBA) emerged.

### 5.5 Body Weight Gain

In general, duration and frequency did not play a role in the effect of exposure to the compound, or when it did, the results were the reverse of what would be biologically meaningful (*e.g.* low dose high duration and high dose low duration). What effects were seen however, appeared to return to normal levels following the recovery period.

### 5.6 Relative Lung Weight

Relative lung weights did appear to be consistently increased in high concentration animals relative to controls following the recovery period. In addition, when the low dose was given in it's highest frequency and duration, relative lung weights were also significantly increased. Following the exposure period, the only significant increase was seen for highest dose frequency and duration combination.

### 5.7 Food Consumption

In general, duration and frequency did not play a role in the effect of exposure to the compound. All animals exhibited significantly decreased food consumption relative to controls.



## 5.8 Pulmonary Function

Overall, there were very few treatment related effects on the pulmonary function variables, and where they did occur, it was generally after adjustment for body weight only. No clear effects of concentration, duration, or frequency were observed, and the absence of significant main effects and the few scattered two-way interactions supports this conclusion. In terms of overall differences from control, some reasonably consistent decreases in RL following the recovery period were observed, and these effects appeared to be independent of body weight. Conversely, TLC and VC appeared to be decreased in post-exposure animals, but these effects only appeared after adjusting for body weight.

## 6 Conclusion

This study has shown that with the exception of pulmonary lavage measures, concentration, duration, and frequency had little if any effect on outcome. Relative to controls, consistent exposure related decreases were observed for ALP, BW, MCV, PLT, MONO, FVC, TLC, and VC, and exposure related increases for NEUT (hematology differential), NEUT (pulmonary lavage), PROTEIN, and FCP, although the effects for the pulmonary function measures were only significant following covariate adjustment for body weight. Following the recovery period consistent decreases in RL, FC, and ALT, and increases in LUNG, TOTVCELL, TOTCELL, and NEUT were observed.

## References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.

- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.

Table 1  
Significant Main Effects and Interactions  
for Graphite Exposure Animals Only

	CONC	FREQ	DUR	C X F	C X D	D X F
Food Consumption				FC <sup>1</sup>		
Pulmonary Function					VCPU <sup>3</sup> CCHORD <sup>3</sup> FES <sup>3</sup> RL <sup>2</sup> CDYN <sup>7</sup> VT <sup>7</sup>	VCPU <sup>2</sup> CCHORD <sup>2</sup>
Clinical Chemistry				ALP <sup>5</sup> BUN <sup>6</sup> TBA <sup>5</sup>	ALP <sup>7</sup> ALT <sup>8</sup> BUN <sup>8</sup> CREA <sup>6</sup> TP <sup>6</sup> ALBG <sup>8</sup> CA <sup>8</sup> TBA <sup>7</sup> PHOS <sup>7</sup> SDH <sup>8</sup>	PHOS <sup>4</sup>
Pulmonary Lavage	MONO <sup>14</sup> NEUT <sup>15</sup> PROTEIN <sup>15</sup>	MONO <sup>12</sup> NEUT <sup>11</sup> PHAGO <sup>12</sup> STDPHAGO <sup>12</sup>	TOTVCELL <sup>13</sup> TOTCELL <sup>13</sup> MONO <sup>14</sup> NEUT <sup>13</sup> PROTEIN <sup>13</sup>		LYMPH <sup>3</sup> STDPHAGO <sup>3</sup> PROTEIN <sup>3</sup> MONO <sup>7</sup>	PHAGO <sup>9</sup> PROTEIN <sup>10</sup>
Body Weight					BW <sup>17</sup>	
Relative Lung Weight			LUNG <sup>11</sup>			
Hematology					MCV <sup>7</sup> MCHC <sup>8</sup>	
			WBC <sup>11</sup> NEUT <sup>11</sup> MONO <sup>11</sup> EOS <sup>11</sup>	NRBC <sup>5</sup>		

<sup>1</sup>high conc, low freq increased in weeks 1 and 5

<sup>2</sup>low freq, high dur increased response

<sup>3</sup>low conc, high dur increased response

<sup>4</sup>low freq, high dur decreased response

<sup>5</sup>low conc, high freq decreased response

<sup>6</sup>low conc, high freq increased response

<sup>7</sup>low conc, high dur decreased response

<sup>8</sup>low conc, high dur increased response

<sup>9</sup>high freq, high dur decreased response

<sup>10</sup>high freq, high dur increased response

<sup>11</sup>high dur increased response

<sup>12</sup>high dur decreased response

<sup>13</sup>high freq increased response

<sup>14</sup>high freq decreased response

<sup>15</sup>high conc increased response

<sup>16</sup>high conc decreased response

<sup>17</sup>high conc, high dur increased response

<sup>18</sup>low conc, low dur increased response

Table 2  
Comparison of Graphite Exposed Animals  
to Filtered Air Controls  
Post Exposure

	MALE				FEMALE			
	LOW CONC		HIGH CONC		LOW CONC		HIGH CONC	
	+	-	+	-	+	-	+	-
Clinical Chemistry		ALP			TRIG	ALP PHOS	TRIG	ALP
Body Weight Gain		AVG		AVG				AVG
Relative Lung Wt								
Hematology						MCV PLT		MCV PLT
Hematology Diff.			NEUT				NEUT	
Lavage <sup>1</sup>	NEUT		NEUT PROT	MONO	NEUT		NEUT PROT	MONO
Pulmonary Function <sup>1</sup>	FOB <sup>2</sup>		FOB <sup>2</sup>	FVC <sup>2</sup> TLC <sup>2</sup> VC <sup>2</sup>	FOB <sup>2</sup>		FOB <sup>2</sup>	FVC <sup>2</sup> TLC <sup>2</sup> VC <sup>2</sup>
		TLC <sup>2</sup> VC <sup>2</sup>				TLC <sup>2</sup> VC <sup>2</sup>		

<sup>1</sup>Males and females not tested separately.

<sup>2</sup>Only significant after body weight adjustment.

Table 3  
Comparison of Graphite Exposed Animals  
to Filtered Air Controls  
Post Recovery

	MALE				FEMALE			
	LOW CONC		HIGH CONC		LOW CONC		HIGH CONC	
	+	-	+	-	+	-	+	-
Clinical Chemistry	CHOL	ALT TP CA	CHOL CREA	ALT	TP TRIG CA	ALT	TRIG	ALT
Body Weight Gain					AVG		AVG	
Relative Lung Wt			RLW		RLW		RLW	
Hematology	PLT WBC		PLT		PLT		PLT	
Hematology Diff.	WBC NEUT MONO							
Food Consumption		AVG TREND		AVG TREND		AVG TREND		AVG TREND
Lavage <sup>1</sup>	TOTVCELL TOTCELL		TOTVCELL TOTCELL NEUT	MONO	TOTVCELL TOTCELL		TOTVCELL TOTCELL NEUT	MONO
Pulmonary Function <sup>1</sup>		FVC <sup>2</sup> RL DLCO <sup>2</sup> VC <sup>2</sup>		RL		FVC <sup>2</sup> RL DLCO <sup>2</sup> VC <sup>2</sup>		RL

<sup>1</sup>Males and females not tested separately.

<sup>2</sup>Only significant after body weight adjustment.

APPENDIX TO PART TWO  
(Statistical Appendix)

SECTIONS

- A. Body Weight Gains
- B. Food Consumption
- C. Lung/Body Weight Ratios
- D. Clinical Chemistry
- E. Hematology
- F. Hematology: Differential Counts
- G. Pulmonary Lavage
- H. Pulmonary Function

PART TWO

APPENDIX

SECTION A. BODY WEIGHT GAINS

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SA/A-1

## 1 L06234 Statistical Report - Body Weight Gains

Multivariate analysis of variance models (Bock, 1975) were used to analyze the body weight gain variables. For the analysis of the exposure period, weekly body weight gain values for weeks 1 through 3 were obtained by summing, for each week, the two weekly body weight gain determinations. A multivariate analysis of variance for repeated measures was then performed, utilizing orthogonal polynomial contrasts, to examine for constant and linear trend effects over the first three weeks of the exposure period. While animals were assessed once during week four in the exposure period, many animals were missing this assessment, and so the analysis was performed simply on the first three weeks. A multivariate analysis of variance was also performed using the two recovery assessments as dependent variables in order to examine any potential changes in body weight gains during the recovery period.

For the analysis of the exposure period body weight gains, the effects of five between-subjects factors were examined: Sex (male or female), Period (designated as exposure or recovery), concentration (control,  $100 \text{ mg/m}^3$ ,  $200 \text{ mg/m}^3$ , or positive control at  $200 \text{ mg/m}^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using a multivariate analysis of variance for repeated measures model which included all five between-subjects factors and all two way interactions of these factors..

The second set of analyses concentrated on examining the differences between the dosed and control animals. First, a one-factor multivariate repeated measures analysis of variance was performed on the males and females separately, with concentration as the grouping factor, augmented by simple contrasts. This allowed a statistical comparison to be made between each group ( $100 \text{ mg/m}^3$ ,  $200 \text{ mg/m}^3$ , and positive control at  $200 \text{ mg/m}^3$ ) and the control group for the male and female animals separately. In order to examine the consistency of any group differences between the levels of duration, a second multivariate repeated measures analysis of variance was performed, again, separately for the male and female animals. In this second analysis, the low and high dose animals were further grouped depending on their duration and frequency of exposure. In this way, again using a one-factor multivariate repeated measures analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration and frequency subgroups.

For the analysis of the recovery period body weight gains, only the effects of four of the five between-subjects factors were examined: Sex (male or female), concentration (control,  $100 \text{ mg/m}^3$ ,  $200 \text{ mg/m}^3$ , or positive control at  $200 \text{ mg/m}^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). Again, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using a multivariate analysis of variance model which included all four between-subjects factors and all two way interactions involving concentration.

The second set of analyses concentrated on examining the differences between the dosed and control animals. First, a one-factor multivariate analysis of variance was performed on the males and females separately, with concentration as the grouping factor, augmented by simple contrasts. This allowed a statistical comparison to be made between each group ( $100 \text{ mg/m}^3$ ,  $200 \text{ mg/m}^3$ , and positive control at  $200 \text{ mg/m}^3$ ) and the control group for the male and female animals separately. In order to examine the consistency of any group differences between the levels of duration, a second multivariate analysis of variance was performed, again, separately for the male and female animals. In this second analysis, the low and high dose animals were further grouped depending



on their duration and frequency of exposure. In this way, again using a one-factor multivariate repeated measures analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration and frequency subgroups.

### 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance for repeated measurements yielded a significant concentration by duration interaction in terms of both the constant ( $p < .001$ ), and the the linear trend over time ( $p < .007$ ). This effect was largely due to larger body weight gains in 4 hr versus 1 hr high dose animals, but the reverse in low dose animals (see Table 2). As expected, weight gains for males were larger than females ( $p < .001$ ), see Table 3. In addition, a significant main effect of duration was also found ( $p < .04$ ), which was due to 4 hr decreases on week 4 (see Table 4).

In terms of the recovery period, a main effect of sex ( $p < .001$ ) was observed, with again males gaining more than females (see Table 5).

All other main effects and two way interactions were observed to be non-significant by the multivariate test.

### 1.2 Analysis of All Animals - Comparison to Controls

In the first set of analyses, concentration differences within males and females were examined. The significant differences that were observed are listed in Table 6, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Tables 7 and 8. For male exposure animals, both low, high dose and positive controls were significantly decreased relative to controls. Following the recovery period, there were no significant differences in body weight gains for male animals. For female exposure animals, high dose and positive controls were significantly decreased relative to controls. Following the recovery period, female low and high dose animals in fact had increased weight gains relative to controls.

In the second set of analyses, concentration differences were again examined for males and females, however, in order to also examine the effects of frequency and duration, the low and high dose animals were further divided into subgroups depending on their level of frequency and duration. The significant differences that were observed for the exposure animals are listed in Table 9, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 10, while corresponding statistics for the recovery period are given in Tables 11 and 12. Inspection of Tables 9 and 10 revealed that (a) both male and female positive controls had decreased weight gains relative to controls, (b) low dose high duration males and females had decreased weight gains relative to controls, and (c) high dose low duration males and females had decreased weight gains relative to controls. These differences did not increase in a linear fashion over time.

Inspection of Tables 11 and 12, revealed that all of these decreases either returned to normal levels or were in fact increased relative to controls.

### 1.3 Summary

In general, duration and frequency did not play a role in the effect of exposure to the compound, or when it did, the results were the reverse of what would be biologically meaningful (*e.g.*, low dose high duration and high dose low duration). What effects were seen however, appeared to return to normal levels following the recovery period.

## References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.

TABLE 1  
 L06234 Body Weight Gain Analysis of Graphite Exposed Animals - Exposure Period  
 Means, SDs, and Ns for Period Designation by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Sex)

PERIOD	FREQUENCY	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
Exposure	2 / week							
	mean	3.35	12.08	1.05	11.63	1.55	9.80	2.33
	sd	5.96	4.98	3.26	5.23	2.75	4.53	2.08
	n	60	60	60	60	60	60	3
	4 / week							
	mean	3.67	11.62	4.43	7.58	5.03	4.95	3.23
	sd	4.96	5.27	2.99	4.09	3.12	5.42	9.72
	n	60	60	60	60	60	60	60
	2 / week							
Recovery	mean	2.57	11.57	1.75	10.50	1.02	9.00	2.78
	sd	3.24	5.54	2.63	3.85	2.33	3.54	2.97
	n	60	60	60	60	60	60	60
	4 / week							
	mean	5.10	11.20	4.37	8.73	5.48	6.32	4.65
	sd	3.85	6.66	2.82	5.01	2.29	4.01	2.61
	n	60	60	60	60	60	60	60
	2 / week							
	mean	3.67	11.62	2.90	9.61	3.27	7.52	3.54
OVERALL	sd	4.68	5.62	3.29	4.81	3.31	4.82	6.03
	n	240	240	240	240	240	240	183

TABLE 2  
L06234 Body Weight Gain Analysis of Graphite Exposed Animals - Exposure Period  
Means, SDs, and Ns for Concentration by Duration Subgroups  
(Averaging over Frequency, Sex, and Period Designation)

CONCENTRATION	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
LowConc	1 Hr/Day							
		mean	5.25	12.28	2.70	10.65	3.70	9.08
		sd	3.39	4.96	2.99	4.89	2.75	4.13
		n	60	60	60	60	60	45
	4 Hr/Day							
		mean	1.05	12.43	2.65	9.15	2.63	6.52
		sd	6.35	5.47	2.85	4.54	3.76	6.00
		n	60	60	60	60	60	45
	1 Hr/Day							
		mean	2.53	10.47	3.55	8.73	3.52	6.60
		sd	3.53	7.28	3.44	4.19	3.53	3.90
		n	60	60	60	60	60	48
HighConc	4 Hr/Day							
		mean	5.85	11.28	2.70	9.92	3.23	7.87
		sd	2.98	4.24	3.80	5.44	3.07	4.63
		n	60	60	60	60	60	45
	1 Hr/Day							
		mean	3.67	11.62	2.90	9.61	3.27	7.52
		sd	4.68	5.62	3.29	4.81	3.31	4.82
		n	240	240	240	240	240	183
	OVERALL							
		mean	3.67	11.62	2.90	9.61	3.27	7.52
		sd	4.68	5.62	3.29	4.81	3.31	4.82
		n	240	240	240	240	240	183

TABLE 3  
 L06234 Body Weight Gain Analysis of Graphite Exposed Animals - Exposure Period  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Period Designation)

SEX		3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
Male								
	mean	4.93	15.39	3.29	12.81	4.19	10.07	3.82
	sd	4.87	4.21	3.71	4.04	3.46	5.13	8.14
	n	120	120	120	120	120	120	90
Female								
	mean	2.41	7.84	2.51	6.42	2.35	4.97	3.26
	sd	4.14	4.13	2.77	3.10	2.88	2.68	2.77
	n	120	120	120	120	120	120	93
OVERALL	mean	3.67	11.62	2.90	9.61	3.27	7.52	3.54
	sd	4.68	5.62	3.29	4.81	3.31	4.82	6.03
	n	240	240	240	240	240	240	183

TABLE 4  
 L06234 Body Weight Gain Analysis of Graphite Exposed Animals - Exposure Period  
 Means, SDs, and Ns for Duration Subgroups  
 (Averaging over Concentration, Frequency, Period Designation, and Sex)

DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
1 Hr/Day							
mean	3.89	11.38	3.12	9.69	3.61	7.84	4.55
sd	3.71	6.27	3.23	4.64	3.15	4.19	3.17
n	120	120	120	120	120	120	93
4 Hr/Day							
mean	3.45	11.86	2.68	9.53	2.93	7.19	2.49
sd	5.50	4.91	3.34	5.00	3.43	5.38	7.86
n	120	120	120	120	120	120	90
OVERALL mean	3.67	11.62	2.90	9.61	3.27	7.52	3.54
sd	4.68	5.62	3.29	4.81	3.31	4.82	6.03
n	240	240	240	240	240	240	183

TABLE 5  
 L06234 Body Weight Gain Analysis of Graphite Exposed Animals - Recovery Period  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Duration, and Frequency)

SEX	4DAYWK48	7DAYWK5
Male		
mean	10.67	18.95
sd	3.26	4.91
n	60	60
Female		
mean	5.63	8.62
sd	1.90	2.74
n	60	60
OVERALL mean	8.15	13.78
sd	3.66	6.52
n	120	120

TABLE 6  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Sex*  
(averaging over frequency and duration)

	concentration	significant increases	significant decreases
<i>Male</i>	100 mg/m <sup>3</sup>		1
	200 mg/m <sup>3</sup>		1
	positive control		1
<i>Female</i>	100 mg/m <sup>3</sup>	3	
	200 mg/m <sup>3</sup>	3	1
	positive control		1

*Key for significant effects*

1 = constant effect over time during exposure period  
(post-exposure and post-recovery assay animals)

2 = linear trend over time during exposure period  
(post-exposure and post-recovery assay animals)

3 = 4DAYWK4B, 4 = 7DAYWK5  
(post-recovery assay animals only)

normal text:  $p < .05$ , bold text:  $p < .01$



TABLE 7  
L06234 Body Weight Gain Analysis of All Animals - Exposure Period  
Means, SDs, and Ns for Sex Subgroups  
(Averaging over Frequency and Duration)

SEX	GROUP	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A
Male	Control							
	mean	8.25	12.86	6.40	12.09	6.30	10.79	6.82
	sd	2.63	3.76	3.04	3.47	3.06	3.94	3.86
	n	79	80	80	80	80	80	80
	LowConc							
	mean	4.43	16.23	3.07	13.13	4.18	10.00	1.62
	sd	5.53	3.90	3.24	3.97	3.66	6.35	10.70
	n	60	60	60	60	60	60	45
	HighConc							
	mean	5.43	14.55	3.52	12.48	4.20	10.13	6.02
	sd	4.09	4.37	4.14	4.12	3.27	3.58	3.10
	n	60	60	60	60	60	60	45
	PosCont							
	mean	6.15	13.38	3.70	10.40	5.92	7.30	6.68
	sd	7.51	3.14	6.62	7.58	2.85	5.66	4.65
	n	40	40	40	40	40	40	40
Female	Control							
	mean	3.45	7.73	1.36	6.60	4.84	4.79	4.24
	sd	2.16	2.47	7.41	6.02	3.29	2.43	2.48
	n	80	80	80	80	80	80	80
	LowConc							
	mean	1.87	8.48	2.28	6.67	2.15	5.60	2.80
	sd	5.18	2.97	2.49	2.96	2.60	2.49	2.78
	n	60	60	60	60	60	60	45
	HighConc							
	mean	2.95	7.20	2.73	6.17	2.55	4.33	3.69
	sd	2.67	4.97	3.02	3.25	3.14	2.75	2.71
	n	60	60	60	60	60	60	48
	PosCont							
	mean	2.60	6.29	3.82	4.43	3.90	3.80	3.05
	sd	4.45	6.39	5.05	3.65	3.22	2.30	4.17
	n	40	38	38	40	40	40	40

TABLE 8  
 L06234 Body Weight Gain Analysis of All Animals - Recovery Period  
 Means, SDs, and Ns for Sex Subgroups  
 (averaging over frequency and duration)

SEX	GROUP	4DAYWK48	7DAYWK5
Male	Control		
		mean	17.95
		sd	5.23
		n	40
	LowConc		
		mean	18.10
		sd	5.33
		n	30
	HighConc		
		mean	19.80
		sd	4.37
		n	30
	PosCont		
		mean	17.24
		sd	6.63
		n	21
Female	Control		
		mean	8.55
		sd	5.51
		n	40
	LowConc		
		mean	8.03
		sd	2.19
		n	30
	HighConc		
		mean	9.20
		sd	3.12
		n	30
	PosCont		
		mean	6.85
		sd	7.31
		n	20

TABLE 9  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
Exposure Period

*Broken Down by Frequency, Duration, and Sex*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	M		
100 mg/m <sup>3</sup>	2	1	F		
100 mg/m <sup>3</sup>	2	4	M		1
100 mg/m <sup>3</sup>	2	4	F		1
100 mg/m <sup>3</sup>	4	1	M		
100 mg/m <sup>3</sup>	4	1	F		
100 mg/m <sup>3</sup>	4	4	M		1
100 mg/m <sup>3</sup>	4	4	F		
200 mg/m <sup>3</sup>	2	1	M		1
200 mg/m <sup>3</sup>	2	1	F		1
200 mg/m <sup>3</sup>	2	4	M		
200 mg/m <sup>3</sup>	2	4	F		
200 mg/m <sup>3</sup>	4	1	M		1
200 mg/m <sup>3</sup>	4	1	F		
200 mg/m <sup>3</sup>	4	4	M		
200 mg/m <sup>3</sup>	4	4	F		
positive control			M		1
positive control			F		1

*Key for significant effects*

1 = constant effect over time during exposure period  
(post-exposure and post-recovery assay animals)

2 = linear trend over time during exposure period  
(post-exposure and post-recovery assay animals)

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 10  
L06234 Body Weight Gain Analysis of All Animals - Exposure Period  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A			
Male	Control	4 / week	4 Hr/Day	mean	8.25	12.86	6.40	12.09	6.30	10.79	6.82		
				sd	2.63	3.76	3.04	3.47	3.06	3.94	3.86		
				n	79	80	80	80	80	80	80		
		LowConc	2 / week	1 Hr/Day	mean	7.20	16.47	2.07	15.80	3.87	13.93	.	
					sd	3.17	1.85	2.71	2.83	2.61	2.89	.	
					n	15	15	15	15	15	15	0	
				4 / week	4 Hr/Day	mean	1.93	15.40	2.60	14.07	.13	12.07	3.80
						sd	2.60	1.50	2.26	3.65	1.88	2.58	2.70
						n	15	15	15	15	15	15	15
				4 / week	1 Hr/Day	mean	7.07	16.67	4.33	12.20	6.07	10.13	5.60
						sd	1.98	3.37	3.75	4.02	2.28	2.42	2.53
						n	15	15	15	15	15	15	15
				4 / week	4 Hr/Day	mean	1.53	16.40	3.27	10.47	6.67	3.87	-4.53
						sd	8.77	6.79	3.83	3.42	3.58	9.37	16.84
						n	15	15	15	15	15	15	15

TABLE 10  
L06234 Body Weight Gain Analysis of All Animals - Exposure Period  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A	
Male	HighConc	2 / week	1 Hr/Day	mean	.87	15.73	2.53	12.27	1.80	10.60	5.47
				sd	3.16	6.73	3.62	3.28	3.12	3.09	2.45
				n	15	15	15	15	15	15	15
		4 Hr/Day	mean	8.73	14.27	-.87	16.20	2.67	13.53	.	
			sd	3.20	3.45	3.64	3.69	2.41	2.64	.	
			n	15	15	15	15	15	15	0	
		4 / week	1 Hr/Day	mean	5.67	13.27	6.33	9.47	6.40	7.73	7.67
				sd	3.33	3.75	2.29	3.07	2.44	3.13	3.46
				n	15	15	15	15	15	15	15
		4 Hr/Day	mean	6.47	14.93	6.07	12.00	5.93	8.67	4.93	
			sd	1.96	2.37	1.91	3.61	2.55	2.55	2.79	
			n	15	15	15	15	15	15	15	
	PosCont	4 / week	4 Hr/Day	mean	6.15	13.38	3.70	10.40	5.92	7.30	6.68
				sd	7.51	3.14	6.62	7.58	2.85	5.66	4.65
				n	40	40	40	40	40	40	40

TABLE 10  
L06234 Body Weight Gain Analysis of All Animals - Exposure Period  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A		
Female	Control	4 / week	4 Hr/Day	mean	3.45	7.73	1.36	6.60	4.84	4.79	4.24	
				sd	2.16	2.47	7.41	6.02	3.29	2.43	2.48	
				n	80	80	80	80	80	80	80	
		LowConc	2 / week	1 Hr/Day	mean	3.20	8.07	1.07	8.20	2.00	6.93	.40
					sd	3.95	2.34	1.79	1.70	1.65	2.05	1.55
					n	15	15	15	15	15	15	15
				4 Hr/Day	mean	-2.93	10.07	1.13	6.80	-.33	6.27	.
					sd	4.59	3.06	2.17	2.48	1.80	1.58	.
					n	15	15	15	15	15	15	0
		4 / week	1 Hr/Day	mean	3.53	7.93	3.33	6.40	2.87	5.33	4.27	
				sd	1.88	2.22	2.53	4.14	2.67	2.64	1.83	
				n	15	15	15	15	15	15	15	
				4 Hr/Day	mean	3.67	7.87	3.60	5.27	4.07	3.87	3.73
					sd	6.30	3.72	2.41	2.43	2.05	2.59	3.03
					n	15	15	15	15	15	15	15

TABLE 10  
L06234 Body Weight Gain Analysis of All Animals - Exposure Period  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	3DAYWK1A	4DAYWK1B	3DAYWK2A	4DAYWK2B	3DAYWK3A	4DAYWK3B	3DAYWK4A	
Female	HighConc	2 / week	1 Hr/Day	mean	.40	7.53	1.87	7.73	.00	5.47	2.33
				sd	3.14	5.26	3.70	2.25	1.56	1.36	2.08
				n	15	15	15	15	15	15	3
		4 Hr/Day	mean	4.27	7.07	.80	7.47	.13	6.40	1.47	
			sd	2.22	2.69	2.21	1.68	1.98	2.80	2.17	
			n	15	15	15	15	15	15	15	
	4 / week	1 Hr/Day	mean	3.20	5.33	3.47	5.47	5.87	2.60	4.33	
			sd	1.66	7.78	2.29	4.72	1.81	2.44	1.80	
			n	15	15	15	15	15	15	15	
		4 Hr/Day	mean	3.93	8.87	4.80	4.00	4.20	2.87	5.53	
			sd	1.62	1.41	2.14	2.07	2.11	2.17	2.50	
			n	15	15	15	15	15	15	15	
PosCont	4 / week	4 Hr/Day	mean	2.60	6.29	3.82	4.43	3.90	3.80	3.05	
			sd	4.45	6.39	5.05	3.65	3.22	2.30	4.17	
			n	40	38	38	40	40	40	40	

TABLE 11  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
Recovery Period

*Broken Down by Frequency, Duration, and Sex*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	F	<b>3</b>	
100 mg/m <sup>3</sup>	2	4	M		
100 mg/m <sup>3</sup>	4	1	M		
100 mg/m <sup>3</sup>	4	4	F	<b>3</b>	
200 mg/m <sup>3</sup>	2	1	M		
200 mg/m <sup>3</sup>	2	4	F	<b>3</b>	
200 mg/m <sup>3</sup>	4	1	F	<b>3</b>	
200 mg/m <sup>3</sup>	4	4	M		
positive control			M		
positive control			F		

*Key for significant effects*

3 = 4DAYWK4B, 4 = 7DAYWK5

(post-recovery assay animals only)

normal text:  $p < .05$ , bold text:  $p < .01$



TABLE 12  
L06234 Body Weight Gain Analysis of All Animals - Recovery Period  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	4DAYWK48	7DAYWK5
Male	Control	4 / week	4 Hr/Day		
				mean	17.95
				sd	5.23
				n	40
	LowConc	2 / week	4 Hr/Day		
				mean	19.20
				sd	5.94
				n	15
		1 / week	1 Hr/Day		
				mean	17.00
				sd	4.58
				n	15
	HighConc	2 / week	1 Hr/Day		
				mean	18.40
				sd	3.62
				n	15
		4 / week	4 Hr/Day		
				mean	21.20
				sd	4.71
				n	15
	PosCont	4 / week	4 Hr/Day		
				mean	18.10
				sd	5.47
				n	20

TABLE 12  
L06234 Body Weight Gain Analysis of All Animals - Recovery Period  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	4DAYWK4B	7DAYWK5
Female	Control	4 / week	4 Hr/Day		
				mean	8.77
				sd	5.40
				n	39
	LowConc	2 / week	1 Hr/Day		
				mean	7.33
				sd	1.84
				n	15
		4 / week	4 Hr/Day		
				mean	8.73
				sd	2.34
				n	15
	HighConc	2 / week	4 Hr/Day		
				mean	8.80
				sd	3.00
				n	15
		4 / week	1 Hr/Day		
				mean	9.60
				sd	3.29
				n	15
	PosCont	4 / week	4 Hr/Day		
				mean	6.85
				sd	7.31
				n	20

PART TWO

APPENDIX

SECTION B. FOOD CONSUMPTION

IIT RESEARCH INSTITUTE

SA/B-1

# 1 L06234 Statistical Report - Food Consumption

Multivariate analysis of variance models for repeated measures (Bock, 1975) were used to analyze the food consumption variables. In these analyses, polynomial contrasts were utilized to test for constant effects across time as well as for linear trends across time. Although two food consumption assessments were obtained per week, these two values were averaged to yield an average food consumption value for each of the five weeks. Prior to analysis, log transformation of the data was performed on these weekly values to better approximate the normality assumption of the statistical model. The effects of four factors were examined in these analyses: Sex (male or female), concentration (control, 100  $mg/m^3$ , 200  $mg/m^3$ , or positive control at 200  $mg/m^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design, including the effect of period (exposure or recovery), in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. Also, since food consumption was assessed only in the animals designated as recovery animals, not all cells of the fractional factorial were present for the food consumption analyses. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using the repeated measures multivariate analysis of variance model which included all four factors and all interactions involving concentration. The second set of analyses concentrated on examining the differences between the dosed and control animals. These analyses were performed separately for males and females, in order to examine group differences while controlling for the effect of sex. First, a one-factor multivariate analysis of variance for repeated measures was performed on the weekly food consumption values, with concentration as the grouping factor, augmented by simple contrasts which allowed a statistical comparison to be made between each group (100  $mg/m^3$ , 200  $mg/m^3$ , and positive control at 200  $mg/m^3$ ) and the control group. In order to examine the consistency of any group differences between the levels of frequency and duration, a second analysis of variance was performed. In this second analysis, the low and high dose animals were further grouped depending on their duration and frequency of exposure. In this way, again using a one-factor multivariate analysis of variance model for repeated measures augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration and frequency subgroups.

## 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance for repeated measurements yielded a significant concentration by frequency interaction in terms of the linear trend over time ( $p < .02$ ). This effect was largely due to increased food consumption in high dose 2/week animals in weeks 1 and 5 (see Table 1). In addition, males had higher food consumption than females ( $p < .001$ ), see Table 2. All other main effects and two way interactions were observed to be non-significant by the multivariate test.

## 1.2 Analysis of All Animals - Comparison to Controls

In the first set of analyses, concentration differences within males and females were examined. The significant differences that were observed are listed in Table 3, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 4. All treated and positive controls groups were significantly decreased relative to controls, and these decreases were generally linearly increasing over the course of the study.

In the second set of analyses, concentration differences were again examined for males and females, however, in order to also examine the effects of frequency and duration, the low and

high dose animals were further divided into subgroups depending on their level of frequency and duration. The significant differences that were observed for the exposure animals are listed in Table 5, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 6. All treated and positive controls groups regardless of frequency or duration, were significantly decreased relative to controls, and these decreases were generally linearly increasing over the course of the study.

### 1.3 Summary

In general, duration and frequency did not play a role in the effect of exposure to the compound. All animals exhibited significantly decreased food consumption relative to controls.

### References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.

TABLE 1  
L06234 Average Daily Food Consumption Analysis of Graphite-Exposed Animals  
Means, SDs, and Ns for Concentration by Frequency Subgroups across Time  
(Averaging over Duration and Sex)

CONCENTRATION	FREQUENCY	FCWEEK1	FCWEEK2	FCWEEK3	FCWEEK4	FCWEEK5
LowConc	2 / week					
	mean	18.01	17.53	16.07	15.87	15.78
	sd	2.57	1.93	2.39	2.96	2.46
	n	18	18	18	18	18
	4 / week					
	mean	18.52	16.93	15.00	15.82	15.70
	sd	1.21	2.22	2.48	2.23	2.25
	n	18	18	18	18	18
HighConc	2 / week					
	mean	19.38	17.51	15.55	15.79	16.16
	sd	3.23	1.83	1.90	2.84	2.84
	n	18	18	18	18	18
	4 / week					
	mean	17.68	16.63	16.01	15.82	15.73
	sd	1.83	2.68	2.75	2.97	2.63
	n	18	18	18	18	18
OVERALL	mean	18.40	17.15	15.66	15.82	15.84
	sd	2.38	2.18	2.39	2.71	2.51
	n	72	72	72	72	72

TABLE 1  
L06234 Average Daily Food Consumption Analysis of Graphite-Exposed Animals  
Means, SDs, and Ns for Concentration by Frequency Subgroups across Time  
(Averaging over Duration and Sex)

CONCENTRATION	FREQUENCY	FCWEEK1A	FCWEEK1B	FCWEEK2A	FCWEEK2B	FCWEEK3A	FCWEEK3B	FCWEEK4A	FCWEEK4B	FCWEEK5A	FCWEEK5B		
LowConc	2 / week												
		mean	16.44	17.68	18.34	16.71	16.59	15.79	16.88	15.35	16.43	15.14	
		sd	1.26	2.97	1.52	2.56	2.58	2.10	2.72	3.13	3.01	2.04	
		n	9	18	18	18	18	17	16	18	18	18	
	4 / week												
		mean	19.14	18.49	17.30	16.56	15.03	14.97	15.46	15.97	15.91	15.49	
		sd	1.53	1.30	2.53	1.94	2.60	2.45	2.22	2.18	2.28	2.28	
		n	7	18	18	18	18	18	17	18	18	18	
	HighConc	2 / week											
			mean	20.03	18.73	18.09	16.93	15.29	15.80	15.81	13.33	16.52	15.79
			sd	3.24	3.33	1.89	1.89	2.20	1.81	2.79	1.56	3.41	2.36
			n	18	18	18	18	18	18	18	9	18	18
4 / week													
		mean	18.08	17.29	16.88	16.37	16.11	15.92	15.72	15.92	15.71	15.76	
		sd	2.34	1.52	2.34	3.11	2.64	2.92	3.44	2.87	2.54	2.77	
		n	18	18	18	18	18	18	18	18	18	18	
OVERALL	mean	18.61	18.05	17.65	16.64	15.76	15.62	15.95	15.40	16.14	15.54		
	sd	2.74	2.47	2.15	2.39	2.53	2.34	2.83	2.71	2.80	2.34		
	n	52	72	72	72	72	71	69	63	72	72		

TABLE 2  
 L06234 Average Daily Food Consumption Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups across Time  
 (Averaging over Concentration, Duration, and Frequency)

SEX	FCWEEK1	FCWEEK2	FCWEEK3	FCWEEK4	FCWEEK5
Male					
mean	20.21	18.96	17.75	18.19	18.08
sd	1.80	1.03	1.17	1.48	1.23
n	36	36	36	36	36
Female					
mean	16.59	15.33	13.57	13.46	13.60
sd	1.23	1.33	1.09	1.07	.96
n	36	36	36	36	36
OVERALL mean	18.40	17.15	15.66	15.82	15.84
sd	2.38	2.18	2.39	2.71	2.51
n	72	72	72	72	72



TABLE 2  
L06234 Average Daily Food Consumption Analysis of Graphite-Exposed Animals  
Means, SDs, and Ns for Sex Subgroups across Time  
(Averaging over Concentration, Duration, and Frequency)

SEX		FCWEEK1A	FCWEEK1B	FCWEEK2A	FCWEEK2B	FCWEEK3A	FCWEEK3B	FCWEEK4A	FCWEEK4B	FCWEEK5A	FCWEEK5B
Male											
	mean	20.83	19.82	19.27	18.66	17.96	17.54	18.20	18.13	18.64	17.53
	sd	2.16	1.90	1.12	1.19	1.24	1.30	2.00	1.31	1.40	1.37
	n	25	36	36	36	36	36	35	27	36	36
Female											
	mean	16.56	16.28	16.04	14.63	13.56	13.64	13.62	13.36	13.65	13.56
	sd	1.17	1.51	1.66	1.33	1.25	1.27	1.19	1.31	1.09	1.05
	n	27	36	36	36	36	35	34	36	36	36
OVERALL	mean	18.61	18.05	17.65	16.64	15.76	15.62	15.95	15.40	16.14	15.54
	sd	2.74	2.47	2.15	2.39	2.53	2.34	2.83	2.71	2.80	2.34
	n	52	72	72	72	72	71	69	63	72	72

TABLE 3  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Sex*  
(averaging over frequency and duration)

	concentration	significant increases	significant decreases
<i>Male</i>	100 <i>mg/m</i> <sup>3</sup>		1,2
	200 <i>mg/m</i> <sup>3</sup>		1,2
	positive control		1,2
<i>Female</i>	100 <i>mg/m</i> <sup>3</sup>		1,2
	200 <i>mg/m</i> <sup>3</sup>		1,2
	positive control		1,2

*Key for significant effects*

1 = constant effect over time

2 = linear trend over time

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 4  
L06234 Average Daily Food Consumption Analysis  
Means, SDs, and Ns for Sex Subgroups across Time  
(averaging over frequency and duration)

SEX	GROUP	FCWEEK1	FCWEEK2	FCWEEK3	FCWEEK4	FCWEEK5	
Male	Control	mean	21.48	20.90	20.51	20.84	20.69
		sd	1.67	1.40	1.32	1.08	1.40
		n	18	18	18	18	18
	LowConc	mean	19.68	19.02	17.66	18.08	17.78
		sd	1.40	.96	1.00	1.34	1.27
		n	18	18	18	18	18
	HighConc	mean	20.74	18.91	17.83	18.30	18.38
		sd	2.03	1.12	1.35	1.64	1.14
		n	18	18	18	18	18
	PosCont	mean	19.56	18.33	16.78	17.09	17.97
		sd	1.49	1.65	1.50	1.12	1.23
		n	12	12	12	12	12
Female	Control	mean	16.56	15.92	15.15	15.39	15.41
		sd	1.06	.96	.80	1.12	.95
		n	18	18	18	18	18
	LowConc	mean	16.85	15.43	13.40	13.61	13.70
		sd	1.43	1.04	1.35	1.15	.82
		n	18	18	18	18	18
	HighConc	mean	16.33	15.23	13.73	13.31	13.51
		sd	.95	1.59	.75	.99	1.10
		n	18	18	18	18	18
	PosCont	mean	16.15	14.81	13.40	13.33	12.85
		sd	1.37	.82	1.04	1.03	1.07
		n	12	12	12	12	12

TABLE 4  
L06234 Average Daily Food Consumption Analysis  
Means, SDs, and Ns for Sex Subgroups across Time  
(Averaging over Frequency and Duration)

SEX	GROUP	FCWEEK1A	FCWEEK1B	FCWEEK2A	FCWEEK2B	FCWEEK3A	FCWEEK3B	FCWEEK4A	FCWEEK4B	FCWEEK5A	FCWEEK5B
Male	Control										
	mean	21.66	21.31	21.03	20.76	20.41	20.61	20.87	20.82	20.41	20.97
	sd	1.59	1.84	1.72	1.23	1.47	1.44	1.32	1.39	1.72	1.29
	n	18	18	18	18	18	18	18	18	18	18
	LowConc										
	mean	19.14	19.65	19.51	18.53	18.10	17.23	18.15	17.94	18.48	17.09
	sd	1.53	1.50	1.07	1.04	1.11	1.09	1.55	1.32	1.37	1.44
	n	7	18	18	18	18	18	17	18	18	18
	HighConc										
	mean	21.49	19.98	19.03	18.78	17.81	17.85	18.25	18.49	18.79	17.97
	sd	2.03	2.27	1.14	1.35	1.37	1.44	2.39	1.28	1.46	1.17
	n	18	18	18	18	18	18	18	9	18	18
PosCont											
mean	19.33	19.78	18.49	18.18	16.92	16.65	17.05	17.13	18.24	17.71	
sd	2.21	1.40	1.71	1.74	1.64	1.46	1.51	.89	1.28	1.26	
n	12	12	12	12	12	12	12	12	12	12	
Female	Control										
	mean	16.84	16.44	16.00	15.84	14.74	15.57	15.34	15.40	15.37	15.46
	sd	1.09	1.22	1.32	.92	1.24	.96	1.08	1.33	1.11	1.05
	n	14	18	18	18	18	18	17	18	18	18
	LowConc										
	mean	16.44	16.52	16.14	14.73	13.52	13.40	14.02	13.37	13.86	13.54
	sd	1.26	1.86	1.47	1.27	1.54	1.37	1.36	1.38	1.09	.80
	n	9	18	18	18	18	17	16	18	18	18
	HighConc										
	mean	16.62	16.04	15.94	14.52	13.59	13.87	13.27	13.34	13.44	13.57
	sd	1.15	1.06	1.87	1.42	.91	1.16	.91	1.27	1.08	1.28
	n	18	18	18	18	18	18	18	18	18	18
PosCont											
mean	17.01	15.51	14.75	14.68	13.56	13.23	13.43	13.23	12.46	13.25	
sd	2.60	1.42	1.33	1.60	1.42	.89	1.12	1.18	1.33	1.20	
n	11	12	11	12	12	12	12	12	12	12	

TABLE 5  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Broken Down by Frequency, Duration, and Sex*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	F		<b>1,2</b>
100 mg/m <sup>3</sup>	2	4	M		<b>1,2</b>
100 mg/m <sup>3</sup>	4	1	M		<b>1</b>
100 mg/m <sup>3</sup>	4	4	F		<b>1,2</b>
200 mg/m <sup>3</sup>	2	1	M		<b>1,2</b>
200 mg/m <sup>3</sup>	2	4	F		<b>1,2</b>
200 mg/m <sup>3</sup>	4	1	F		<b>1,2</b>
200 mg/m <sup>3</sup>	4	4	M		<b>1</b>
positive control			M		<b>1,2</b>
positive control			F		<b>1,2</b>

*Key for significant effects*

1 = constant effect over time

2 = linear trend over time

normal text:  $p < .05$ , **bold text:**  $p < .01$

TABLE 6  
L06234 Average Daily Food Consumption Analysis  
Means, SDs, and Ns for All Subgroups across Time

SEX	GROUP	FREQUENCY	DURATION	FCWEEK1	FCWEEK2	FCWEEK3	FCWEEK4	FCWEEK5
Male	Control	4 / week	4 Hr/Day					
				mean	21.48	20.90	20.51	20.84
				sd	1.67	1.40	1.32	1.08
				n	18	18	18	18
	LowConc	2 / week	4 Hr/Day					
				mean	20.23	19.16	18.09	18.51
				sd	1.45	.89	.76	1.32
				n	9	9	9	9
		4 / week	1 Hr/Day					
				mean	19.12	18.88	17.23	17.65
				sd	1.17	1.07	1.07	1.28
				n	9	9	9	9
	HighConc	2 / week	1 Hr/Day					
				mean	22.16	18.73	17.21	18.22
				sd	1.81	1.30	1.02	1.54
				n	9	9	9	9
		4 / week	4 Hr/Day					
				mean	19.31	19.08	18.46	18.38
				sd	.95	.95	1.39	1.83
				n	9	9	9	9
	PosCont	4 / week	4 Hr/Day					
				mean	19.56	18.33	16.78	17.09
				sd	1.49	1.65	1.50	1.12
				n	12	12	12	12

TABLE 6  
L06234 Average Daily Food Consumption Analysis  
Means, SDs, and Ns for All Subgroups across Time

SEX	GROUP	FREQUENCY	DURATION	FCWEEK1	FCWEEK2	FCWEEK3	FCWEEK4	FCWEEK5
Female	Control	4 / week	4 Hr/Day					
				mean	16.56	15.92	15.15	15.39
				sd	1.06	.96	.80	1.12
				n	18	18	18	18
	LowConc	2 / week	1 Hr/Day					
				mean	15.78	15.89	14.04	13.22
				sd	.91	1.06	1.52	1.05
				n	9	9	9	9
		4 / week	4 Hr/Day					
				mean	17.92	14.97	12.77	13.99
				sd	.96	.84	.82	1.17
				n	9	9	9	9
	HighConc	2 / week	4 Hr/Day					
				mean	16.60	16.29	13.89	13.36
				sd	1.22	1.44	.68	1.22
				n	9	9	9	9
		4 / week	1 Hr/Day					
				mean	16.06	14.17	13.57	13.25
				sd	.52	.89	.82	.78
				n	9	9	9	9
	PosCont	4 / week	4 Hr/Day					
				mean	16.15	14.81	13.40	13.33
				sd	1.37	.82	1.04	1.03
				n	12	12	12	12

TABLE 6  
L06234 Average Daily Food Consumption Analysis  
Means, SDs, and Ns for All Subgroups across Time

SEX	GROUP	FREQUENCY	DURATION	FCWEEK1A	FCWEEK1B	FCWEEK2A	FCWEEK2B	FCWEEK3A	FCWEEK3B	FCWEEK4A	FCWEEK4B	FCWEEK5A	FCWEEK5B			
Male	Control	4 / week	4 Hr/Day	mean	21.66	21.31	21.03	20.76	20.41	20.61	20.87	20.82	20.41	20.97		
				sd	1.59	1.84	1.72	1.23	1.47	1.44	1.32	1.39	1.72	1.29		
				n	18	18	18	18	18	18	18	18	18	18		
		LowConc	2 / week	4 Hr/Day	mean	.	20.23	19.48	18.83	18.77	17.42	18.82	18.20	19.14	16.80	
					sd	.	1.45	.92	1.04	.85	.78	1.54	1.33	1.11	1.37	
					n	0	9	9	9	9	9	9	9	9	9	
				4 / week	1 Hr/Day	mean	19.14	19.07	19.53	18.23	17.43	17.03	17.40	17.69	17.81	17.38
						sd	1.53	1.38	1.26	1.00	.95	1.35	1.23	1.34	1.33	1.53
						n	7	9	9	9	9	9	8	9	9	9
	HighConc			2 / week	1 Hr/Day	mean	22.80	21.52	19.08	18.39	17.21	17.20	18.22	.	19.66	17.88
						sd	1.84	2.10	1.42	1.31	1.08	1.16	1.54	.	1.17	1.02
						n	9	9	9	9	9	9	9	0	9	9
				4 / week	4 Hr/Day	mean	20.18	18.44	18.98	19.18	18.41	18.50	18.28	18.49	17.93	18.07
						sd	1.23	1.10	.86	1.34	1.43	1.46	3.13	1.28	1.21	1.36
						n	9	9	9	9	9	9	9	9	9	9
			PosCont	4 / week	4 Hr/Day	mean	19.33	19.78	18.49	18.18	16.92	16.65	17.05	17.13	18.24	17.71
						sd	2.21	1.40	1.71	1.74	1.64	1.46	1.51	.89	1.28	1.26
						n	12	12	12	12	12	12	12	12	12	12
	mean					19.33	19.78	18.49	18.18	16.92	16.65	17.05	17.13	18.24	17.71	
	sd					2.21	1.40	1.71	1.74	1.64	1.46	1.51	.89	1.28	1.26	
	n					12	12	12	12	12	12	12	12	12	12	



TABLE 6  
L06234 Average Daily Food Consumption Analysis  
Means, SDs, and Ns for All Subgroups across Time

SEX	GROUP	FREQUENCY	DURATION	FCWEEK1A	FCWEEK1B	FCWEEK2A	FCWEEK2B	FCWEEK3A	FCWEEK3B	FCWEEK4A	FCWEEK4B	FCWEEK5A	FCWEEK5B	
Female	Control	4 / week	4 Hr/Day	mean	16.84	16.44	16.00	15.84	14.74	15.57	15.34	15.40	15.37	15.46
				sd	1.09	1.22	1.32	.92	1.24	.96	1.08	1.33	1.11	1.05
				n	14	18	18	18	18	18	17	18	18	18
	LowConc	2 / week	1 Hr/Day	mean	16.44	15.12	17.21	14.58	14.41	13.96	14.39	12.50	13.71	13.48
				sd	1.26	1.41	1.10	1.64	1.65	1.46	1.57	.85	1.17	.85
				n	9	9	9	9	9	8	7	9	9	9
		4 / week	4 Hr/Day	mean	.	17.92	15.07	14.88	12.63	12.90	13.73	14.24	14.00	13.60
				sd	.	.96	.90	.82	.71	1.14	1.19	1.28	1.05	.80
				n	0	9	9	9	9	9	9	9	9	9
	HighConc	2 / week	4 Hr/Day	mean	17.26	15.94	17.11	15.48	13.38	14.40	13.39	13.33	13.39	13.70
				sd	1.31	1.30	1.84	1.06	.91	1.09	1.04	1.56	1.14	.97
				n	9	9	9	9	9	9	9	9	9	9
		4 / week	1 Hr/Day	mean	15.98	16.13	14.78	13.57	13.81	13.33	13.16	13.34	13.49	13.44
				sd	.44	.82	.97	1.04	.92	1.01	.80	.99	1.07	1.58
				n	9	9	9	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day	mean	17.01	15.51	14.75	14.68	13.56	13.23	13.43	13.23	12.46	13.25
				sd	2.60	1.42	1.33	1.60	1.42	.89	1.12	1.18	1.33	1.20
				n	11	12	11	12	12	12	12	12	12	12

PART TWO  
APPENDIX

SECTION C. LUNG/BODY WEIGHT RATIOS

IIT RESEARCH INSTITUTE

SA/C-1

# 1 L06234 Statistical Report - Lung/Body Weight

Analysis of variance models (Bock, 1975) were used to analyze the ratio of lung to body weight. Prior to analysis, log transformation of the data was performed of this ratio to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: Sex (male or female), Period (exposure or recovery), concentration (control, 100 mg/m<sup>3</sup>, 200 mg/m<sup>3</sup>, or positive control at 200 mg/m<sup>3</sup>), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using an analysis of variance model which included all five factors and all two way interactions. The second set of analyses concentrated on examining the differences between the dosed and control animals. These analyses were performed separately in each of the four subsamples, defined by period and sex, in order to examine group differences while controlling for the effects of period and sex. First, a one-factor analysis of variance was performed on the lung to body weight ratio, with concentration as the grouping factor, augmented by simple contrasts which allowed a statistical comparison to be made between each group (100 mg/m<sup>3</sup>, 200 mg/m<sup>3</sup>, and positive control at 200 mg/m<sup>3</sup>) and the control group. In order to examine the consistency of any group differences between the levels of frequency and duration, a second analysis of variance was performed. In this second analysis, the low and high dose animals were further grouped depending on their duration and frequency of exposure. In this way, again using a one-factor analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration and frequency subgroups.

## 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance yielded significant interaction effects of sex by frequency ( $p < .017$ ), and significant main effects of sex ( $p < .004$ ), period ( $p < .001$ ), and duration ( $p < .006$ ). All other main effects and two way interactions were observed to be non-significant.

The sex by frequency interaction (Table 1) was due to elevated relative lung weights in females exposed 4 times per week. The main effect of duration (Table 2) was due to elevated relative lung weights in the 4 hr/day animals. The main effect of sex (Table 3) was due to elevated relative lung weights in female animals. The main effect of period (Table 4) was due to elevated relative lung weights in recovery animals.

## 1.2 Analysis of All Animals - Comparison to Controls

In the first set of analyses, concentration differences within each of the four subsamples defined by period and sex were examined. The significant differences that were observed are listed in Table 5, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 6.

Inspection of Table 5 revealed only significant increases in relative lung weights for male and female positive controls in the exposure period and positive controls, high dose males and both low and high dose females following the recovery period.

In the second set of analyses, concentration differences were again examined within each of the four subsamples defined by period and sex. However, in order to also examine the effects of frequency and duration, the low and high dose animals were further divided into subgroups

depending on their level of frequency and duration. The significant differences that were observed for the exposure animals are listed in Table 7, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 8. Similarly, the significant differences that were observed for the recovery animals are listed in Table 9, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 10.

For exposure animals, positive control and female high dose high frequency high duration animals exhibited significantly increased relative lung weights. For recovery animals, significant increases were seen for positive controls, all but the lowest frequency and duration high dose groups, and for female low dose high frequency high duration animals.

### 1.3 Summary

Relative lung weights did appear to be consistently increased in high dose animals relative to controls following the recovery period. In addition, when the low dose was given in it's highest frequency and duration, relative lung weights were also significantly increased. Following the exposure period, the only significant increase was seen for highest dose frequency and duration combination.

### References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.

TABLE 1  
 L06234 Lung/Body Weight Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	FREQUENCY	LUNGBWT
Male	2 / week	
		mean .78
		sd .19
		n 36
	4 / week	
		mean .76
		sd .12
		n 36
Female	2 / week	
		mean .79
		sd .14
		n 36
	4 / week	
		mean .88
		sd .13
		n 36
OVERALL	mean	.80
		sd .15
		n 144

TABLE 2  
 L06234 Lung/Body Weight Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Duration Subgroups  
 (Averaging over Concentration, Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

DURATION	LUNGBWT
1 Hr/Day	
mean	.77
sd	.14
n	72
4 Hr/Day	
mean	.84
sd	.16
n	72
OVERALL mean	.80
sd	.15
n	144

TABLE 3  
 L06234 Lung/Body Weight Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Period)  
 (variables with significant univariate F-test results)

SEX	LUNGBWT
Male	
mean	.77
sd	.16
n	72
Female	
mean	.84
sd	.14
n	72
OVERALL mean	.80
sd	.15
n	144

TABLE 4  
 L06234 Lung/Body Weight Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	LUNGBWT
Post-Exp	
mean	.76
sd	.12
n	72
Post-Rec	
mean	.85
sd	.17
n	72
OVERALL	
mean	.80
sd	.15
n	144



TABLE 5  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Period and Sex*  
(averaging over frequency and duration)

	concentration	significant increases	significant decreases
<i>Male Post-Exposure</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>		
	positive control	1	
<i>Male Post-Recovery</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>	1	
	positive control	1	
<i>Female Post-Exposure</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>		
	positive control	1	
<i>Female Post-Recovery</i>	100 mg/m <sup>3</sup>	1	
	200 mg/m <sup>3</sup>	1	
	positive control	1	
<i>Key for significant effects</i>			
1 = Lung to Body Weight Ratio			
normal text: $p < .05$ , bold text: $p < .01$			

TABLE 6  
L06234 Lung/Body Weight Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	LUNGBWT
Male	Post-Exp	Control	
			mean
			.72
		sd	
			.22
			n
			23
		LowConc	
			mean
			.77
		sd	
			.13
			n
			18
		HighConc	
			mean
			.74
		sd	
			.12
			n
			18
		PosCont	
			mean
			1.05
		sd	
			.12
			n
			12
	Post-Rec	Control	
			mean
			.73
		sd	
			.11
			n
			24
		LowConc	
			mean
			.77
		sd	
			.23
			n
			18
		HighConc	
			mean
			.82
		sd	
			.13
			n
			18
		PosCont	
			mean
			1.32
		sd	
			.13
			n
			12

TABLE 6  
 L06234 Lung/Body Weight Analysis of All Animals  
 Means, SDs, and Ns for Sex by Period Subgroups  
 (Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	LUNGBWT
Female	Post-Exp	Control	
			mean .74
			sd .09
			n 24
		LowConc	
			mean .78
			sd .10
			n 18
		HighConc	
			mean .77
			sd .15
			n 18
		PosCont	
			mean 1.17
			sd .18
			n 12
	Post-Rec	Control	
			mean .78
			sd .13
			n 23
		LowConc	
			mean .88
			sd .16
			n 18
		HighConc	
			mean .91
			sd .12
			n 18
		PosCont	
			mean 1.43
			sd .17
			n 12

TABLE 7  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Exposure Animals by Duration and Frequency*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	M		
100 mg/m <sup>3</sup>	2	4	F		
100 mg/m <sup>3</sup>	4	1	F		
100 mg/m <sup>3</sup>	4	4	M		
200 mg/m <sup>3</sup>	2	1	F		
200 mg/m <sup>3</sup>	2	4	M		
200 mg/m <sup>3</sup>	4	1	M		
200 mg/m <sup>3</sup>	4	4	F	1	
positive control			M	1	
positive control			F	1	

*Key for significant effects*

1 = Lung to Body Weight Ratio

normal text:  $p < .05$ , **bold text:**  $p < .01$

TABLE 8  
L06234 Lung/Body Weight Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	LUNGBWT
Male	Control	4 / week	4 Hr/Day	
				mean .72
				sd .22
				n 23
	LowConc	2 / week	1 Hr/Day	
				mean .75
				sd .15
				n 9
		4 / week	4 Hr/Day	
				mean .78
				sd .11
				n 9
	HighConc	2 / week	4 Hr/Day	
				mean .79
				sd .12
				n 9
		4 / week	1 Hr/Day	
				mean .69
				sd .09
				n 9
	PosCont	4 / week	4 Hr/Day	
				mean 1.05
				sd .12
				n 12

TABLE 8  
L06234 Lung/Body Weight Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	LUNGBWT
Female	Control	4 / week	4 Hr/Day	
				mean .74
				sd .09
				n 24
	LowConc	2 / week	4 Hr/Day	
				mean .75
				sd .10
				n 9
		4 / week	1 Hr/Day	
				mean .80
				sd .11
				n 9
	HighConc	2 / week	1 Hr/Day	
				mean .68
				sd .11
				n 9
		4 / week	4 Hr/Day	
				mean .86
				sd .13
				n 9
	PosCont	4 / week	4 Hr/Day	
				mean 1.17
				sd .18
				n 12

TABLE 9  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Recovery Animals by Duration and Frequency*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	F		
100 mg/m <sup>3</sup>	2	4	M		
100 mg/m <sup>3</sup>	4	1	M		
100 mg/m <sup>3</sup>	4	4	F	1	
200 mg/m <sup>3</sup>	2	1	M		
200 mg/m <sup>3</sup>	2	4	F	1	
200 mg/m <sup>3</sup>	4	1	F	1	
200 mg/m <sup>3</sup>	4	4	M	1	
positive control			M	1	
positive control			F	1	

*Key for significant effects*

1 = Lung to Body Weight Ratio

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 10  
L06234 Lung/Body Weight Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	LUNGBWT
Male	Control	4 / week	4 Hr/Day	
				mean .73
				sd .11
				n 24
	LowConc	2 / week	4 Hr/Day	
				mean .80
				sd .29
				n 9
		4 / week	1 Hr/Day	
				mean .74
				sd .14
				n 9
	HighConc	2 / week	1 Hr/Day	
				mean .80
				sd .18
				n 9
		4 / week	4 Hr/Day	
				mean .83
				sd .07
				n 9
	PosCont	4 / week	4 Hr/Day	
				mean 1.32
				sd .13
				n 12



TABLE 10  
L06234 Lung/Body Weight Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	LUNGBWT
Female	Control	4 / week	4 Hr/Day	
				mean .78
				sd .13
				n 23
	LowConc	2 / week	1 Hr/Day	
				mean .80
				sd .12
				n 9
		4 / week	4 Hr/Day	
				mean .95
				sd .16
				n 9
	HighConc	2 / week	4 Hr/Day	
				mean .91
				sd .15
				n 9
		4 / week	1 Hr/Day	
				mean .91
				sd .09
				n 9
	PosCont	4 / week	4 Hr/Day	
				mean 1.43
				sd .17
				n 12

PART TWO  
APPENDIX

SECTION D. CLINICAL CHEMISTRY

MT RESEARCH INSTITUTE

SA/D-1

# 1 L06234 Statistical Report - Clinical Chemistry

Multivariate analysis of variance models (Bock, 1975) were used to analyze the clinical chemistry variables: CK, ALP, ALT, BUN, CREA, GLU, TP, ALBG, CHOL, TRIG, CA, TBA, PHOS, and SDH. Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: Sex (male or female), Period (exposure or recovery), concentration (control, 100 mg/m<sup>3</sup>, 200 mg/m<sup>3</sup>, or positive control at 200 mg/m<sup>3</sup>), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using a multivariate analysis of variance model which included all five factors and all two way interactions. The second set of analyses concentrated on examining the differences between the dosed and control animals. These analyses were performed separately in each of the four subsamples, defined by period and sex, in order to examine group differences while controlling for the effects of period and sex. First, a one-factor multivariate analysis of variance was performed on the 14 clinical chemistry variables, with concentration as the grouping factor, augmented by simple contrasts which allowed a statistical comparison to be made between each group (100 mg/m<sup>3</sup>, 200 mg/m<sup>3</sup>, and positive control at 200 mg/m<sup>3</sup>) and the control group. In order to examine the consistency of any group differences between the levels of frequency and duration, a second multivariate analysis of variance was performed. In this second analysis, the low and high dose animals were further grouped depending on their duration and frequency of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration and frequency subgroups.

## 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance yielded significant interaction effects of frequency by duration ( $p < .03$ ), concentration by frequency ( $p < .001$ ), concentration by duration ( $p < .001$ ), sex by frequency ( $p < .001$ ), sex by duration ( $p < .001$ ), sex by concentration ( $p < .001$ ), and sex by period ( $p < .001$ ) by the multivariate test. Significant main effects of sex ( $p < .001$ ) and period ( $p < .001$ ) were also observed. All other main effects (i.e., concentration, duration, and frequency) and two-way interactions were observed to be non-significant by the multivariate test.

The frequency by duration interaction was observed to be significant for PHOS ( $p < .03$ ) only, by the univariate F-tests. The observed means for the subgroups defined by frequency and duration, listed in Table 1, illustrate the nature of the frequency by duration interaction for this variable. For the low frequency, increasing duration was associated with decreased PHOS, whereas the reverse was true for the high frequency.

The concentration by frequency interaction was observed to be significant for ALP ( $p < .03$ ), BUN ( $p < .03$ ), and TBA ( $p < .02$ ), by the univariate F-tests. The observed means for the subgroups defined by concentration and frequency, listed in Table 2, illustrate the nature of the concentration by frequency interaction for these variables. For the low dose, increasing frequency was associated with decreased ALP and TBA, whereas the reverse was true for the high dose. For the low dose, increasing frequency was associated with increased BUN, whereas the reverse was true for the high dose.

The concentration by duration interaction was observed to be significant for ALP ( $p < .03$ ), ALT

( $p < .001$ ), BUN ( $p < .001$ ), CREA ( $p < .02$ ), TP ( $p < .001$ ), ALBG ( $p < .001$ ), CA ( $p < .001$ ), TBA ( $p < .006$ ), PHOS ( $p < .001$ ), and SDH ( $p < .001$ ), by the univariate F-tests. The observed means for the subgroups defined by concentration and duration, listed in Table 3, illustrate the nature of the concentration by duration interaction for these variables. For the low dose, increasing duration was associated with decreased ALP, TBA, and PHOS, whereas the reverse was true for the high dose. For the low dose, increasing frequency was associated with increased ALT, BUN, CREA TP, ALBG, CA, and SDH, whereas the reverse was true for the high dose.

The sex by frequency interaction was observed to be significant for CK ( $p < .001$ ), ALP ( $p < .001$ ), ALT ( $p < .009$ ), CREA ( $p < .001$ ), TBA ( $p < .001$ ), and SDH ( $p < .001$ ), by the univariate F-tests. The observed means for the subgroups defined by sex and frequency, listed in Table 4, illustrate the nature of the interaction for these variables. For males, increasing frequency was associated with decreased ALP, whereas the reverse was true for females. For males, increasing frequency was associated with increased CK, ALP, ALT, CREA, TBA, and SDH, whereas the reverse was true for females.

The sex by duration interaction was observed to be significant for CK ( $p < .006$ ), GLU ( $p < .03$ ), TP ( $p < .001$ ), ALBG ( $p < .001$ ), TBA ( $p < .001$ ), and PHOS ( $p < .001$ ), by the univariate F-tests. The observed means for the subgroups defined by sex and duration, listed in Table 5, illustrate the nature of the interaction for these variables. For males, increasing duration was associated with decreased CK, GLU, and PHOS, whereas the reverse was true for females. For males, increasing duration was associated with increased TP, ALBG, and TBA, whereas the reverse was true for females.

The sex by concentration interaction was observed to be significant for ALP ( $p < .04$ ), TP ( $p < .001$ ), ALBG ( $p < .003$ ), CA ( $p < .001$ ), and PHOS ( $p < .003$ ), by the univariate F-tests. The observed means for the subgroups defined by sex and concentration, listed in Table 6, illustrate the nature of the interaction for these variables. For males, increasing concentration was associated with decreased PHOS, whereas the reverse was true for females. For males, increasing concentration was associated with increased ALP, TP, ALBG, and CA, whereas the reverse was true for females.

The sex by period interaction was observed to be significant for ALBG ( $p < .001$ ), CHOL ( $p < .001$ ), and TRIG ( $p < .02$ ), by the univariate F-tests. The observed means for the subgroups defined by sex and period, listed in Table 7, illustrate the nature of the interaction for these variables. Male exposure animals were elevated relative to all other groups for CHOL. Conversely, female exposure animals were elevated relative to the other groups in terms of CHOL. Finally, male recovery animals had elevated TRIG levels relative to the other three groups.

The main effect of sex was observed to be statistically significant by the univariate test for the variables: ALT, GLU, TP, ALBG, CHOL, TRIG, PHOS, and SDH ( $p < .001$  for all, except GLU  $p < .03$  and ALT  $p < .004$ ). Table 8 which lists the observed means for these variables by sex indicates that males had elevated ALT, GLU, TP, ALBG, TRIG, and PHOS means, and decreased CHOL, and SDH means, relative to females.

The main effect of period was observed to be statistically significant by the univariate test for the variables: ALP, BUN, CHOL, CA, TBA, and PHOS ( $p < .001$  for all, except ALP  $p < .04$  and CHOL  $p < .004$ ). Table 9 which lists the observed means for these variables by period indicates that exposure sacrifice animals had elevated ALP, and CHOL, means, and decreased BUN, CA, TBA, and PHOS means, relative to recovery sacrifice animals.

The main effects of concentration, duration, and frequency were not significant.

## 1.2 Analysis of All Animals - Comparison to Controls

In the first set of analyses, concentration differences within each of the four subsamples defined by period and sex were examined. The significant differences that were observed are listed in Table 10, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 11.

The univariate tests performed on the male exposure animals revealed significant group differences only in terms of ALP ( $p < .001$ ). As Tables 10 and 11 reveal, the low dose group exhibited significantly decreased ALP levels, relative to the control group.

For the female exposure animals, significant group differences were observed on ALP ( $p < .001$ ), TRIG ( $p < .004$ ), CA ( $p < .02$ ), and PHOS ( $p < .04$ ). As indicated in Tables 10 and 11, all groups exhibited significant decreases on ALP, and significant increases on TRIG as compared to controls. Also, the positive control group was significantly decreased in terms of CA, as compared to the control group. PHOS levels were also significantly decreased in low dose and positive control animals relative to controls.

Turning to the results from the recovery animals, significant group differences for the male recovery animals were observed by the univariate tests on ALT ( $p < .001$ ), CREA ( $p < .02$ ), TP ( $p < .005$ ), CHOL ( $p < .03$ ), and CA ( $p < .01$ ). Tables 10 and 11 indicate that the positive control group was significantly elevated on CREA relative to the control group. Low dose animals were significantly elevated on CHOL and significantly decreased on ALP, ALT, TP, and CA relative to controls. High dose animals were significantly elevated on CREA and CHOL and significantly decreased on ALT, relative to controls.

For female recovery animals, significant group differences were observed by the univariate tests on ALT ( $p < .001$ ), TP ( $p < .03$ ), TRIG ( $p < .001$ ), CHOL ( $p < .05$ ), and CA ( $p < .03$ ). Tables 10 and 11 indicate that the positive control group was significantly elevated on TP and TRIG, and significantly decreased on ALT and CHOL relative to the control group. Low dose animals were significantly elevated on TP, TRIG, and CA and significantly decreased on ALT, relative to controls. High dose animals were significantly elevated on TRIG and significantly decreased on ALT, relative to controls.

In the second set of analyses, concentration differences were again examined within each of the four subsamples defined by period and sex. However, in order to also examine the effects of frequency and duration, the low and high dose animals were further divided into subgroups depending on their level of frequency and duration. The significant differences that were observed for the exposure animals are listed in Table 12, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 13. Similarly, the significant differences that were observed for the recovery animals are listed in Table 14, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 15.

The univariate tests performed on the male exposure animals revealed significant group differences in terms of CK ( $p < .01$ ), ALP ( $p < .001$ ), ALT ( $p < .03$ ), GLU ( $p < .02$ ), TP ( $p < .04$ ), ALBG ( $p < .01$ ), PHOS ( $p < .02$ ), and SDH ( $p < .001$ ). As Tables 12 and 13 reveal, no significant differences were observed for the positive control group relative to controls. The low dose group with 2 weekly exposures of 1 hour per day exhibited decreased TP, ALBG, and SDH, relative to the control group. The low dose group with 4 weekly exposures of 4 hours per day exhibited decreased ALP and increased SDH levels relative to controls. The high dose group with 2 weekly exposures of 4 hours per day exhibited decreased CK, ALT, PHOS, and SDH relative to the control group. The high dose group with 4 weekly exposures of 1 hour per day exhibited increased GLU levels relative to the control group.

The univariate tests performed on the female exposure animals revealed significant group dif-

ferences in terms of ALP ( $p < .001$ ), CREA ( $p < .02$ ), TP ( $p < .05$ ), ALBG ( $p < .04$ ), TRIG ( $p < .02$ ), CA ( $p < .001$ ), PHOS ( $p < .001$ ), and SDH ( $p < .02$ ). As Tables 12 and 13 reveal, positive controls were significantly decreased on ALP, TP, CA, and PHOS, and significantly increased on TRIG relative to controls. The low dose group with 2 weekly exposures of 4 hours per day exhibited decreased ALP and PHOS, and increased CREA and TRIG relative to the control group. The low dose group with 4 weekly exposures of 1 hour per day exhibited decreased SDH and increased TRIG levels relative to controls. The high dose group with 2 weekly exposures of 1 hour per day exhibited decreased ALP and PHOS, and increased TRIG relative to the control group. The high dose group with 4 weekly exposures of 4 hours per day exhibited increased TRIG and PHOS, and decreased CREA, TP, ALBG, CA, and SDH levels relative to the control group.

The univariate tests performed on the male recovery animals revealed significant group differences in terms of CK ( $p < .003$ ), ALT ( $p < .001$ ), CREA ( $p < .02$ ), TP ( $p < .001$ ), ALBG ( $p < .001$ ), CHOL ( $p < .03$ ), TRIG ( $p < .006$ ), CA ( $p < .006$ ), TBA ( $p < .001$ ), and PHOS ( $p < .001$ ). As Tables 14 and 15 reveal, the positive control group was significantly increased on CREA and decreased on ALT and ALBG relative to controls. The low dose group with 2 weekly exposures of 4 hours per day exhibited increased ALBG, CHOL, and TRIG, and decreased CK, ALT, TBA, and PHOS relative to the control group. The low dose group with 4 weekly exposures of 1 hour per day exhibited increased PHOS and decreased ALT, TP, ALBG, and CA levels relative to controls. The high dose group with 2 weekly exposures of 1 hour per day exhibited increased TRIG and decreased ALT and TBA relative to controls. The high dose group with 4 weekly exposures of 4 hours per day exhibited increased CREA, CHOL, and TBA levels and decreased ALT, and ALBG levels relative to the control group.

The univariate tests performed on the female recovery animals revealed significant group differences in terms of ALT ( $p < .002$ ), BUN ( $p < .03$ ), TP ( $p < .001$ ), ALBG ( $p < .001$ ), TRIG ( $p < .001$ ), CA ( $p < .04$ ), TBA ( $p < .001$ ), and PHOS ( $p < .007$ ). As Tables 14 and 15 reveal, the positive control group was significantly increased on TPA and TRIG, and decreased on ALT, BUN, and TBA relative to controls. The low dose group with 2 weekly exposures of 1 hour per day exhibited increased TP, TRIG, and CA, and decreased ALT and BUN relative to the control group. The low dose group with 4 weekly exposures of 4 hours per day exhibited increased TRIG and CA and decreased TBA levels relative to controls. The high dose group with 2 weekly exposures of 4 hours per day exhibited increased TRIG and PHOS, and decreased ALT and ALBG relative to controls. The high dose group with 4 weekly exposures of 1 hour per day exhibited increased TP, ALBG, TRIG, and CA, levels and decreased ALT levels relative to the control group.

### 1.3 Summary

Overall, the main effects of concentration, duration, and frequency were not significant, suggesting that when averaged over sex and period, little difference in the levels of these factors (*i.e.*, low or high, 1 or 4 hrs, or 2 or 4 times per week) was observed. Numerous interactions among these factors, however, revealed that the results are not quite so easy to interpret. For example, inspection of Table 10 revealed that little if anything was significant for male exposure sacrifice animals, whereas females exhibited consistently increased TRIG levels and decreased ALP and PHOS levels. When broken down by frequency and duration (Table 12), it appeared that females again had consistently elevated TRIG levels regardless of concentration, frequency, or duration, and both male and females exhibited decreased SDH levels for both concentrations and various combinations of frequency and duration. In addition, reasonably consistent decreases in ALP and PHOS were also observed. Following the recovery period the effects on TRIG remained unchanged, but SDH, ALP, and PHOS appeared to return to normal levels. However, after the recovery period, several other consistent

increases (TP, CA) and decreases (ALT, ALBG, and TBA) emerged.

## References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.

TABLE 1  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Frequency by Duration Subgroups  
 (Averaging over Concentration, Period, and Sex)  
 (variables with significant univariate F-test results)

FREQUENCY	DURATION	PHOS
2 / week	1 Hr/Day	
		mean 8.06
		sd 1.57
		n 35
	4 Hr/Day	
		mean 7.60
		sd 1.30
		n 36
4 / week	1 Hr/Day	
		mean 7.87
		sd 1.58
		n 36
	4 Hr/Day	
		mean 8.07
		sd 1.00
		n 36
OVERALL	mean	7.90
	sd	1.38
	n	143



TABLE 2  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Frequency Subgroups  
 (Averaging over Duration, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	FREQUENCY	ALP	BUN	TBA
LowConc	2 / week			
	mean	433.47	17.84	18.21
	sd	49.53	1.92	5.32
	n	36	36	36
	4 / week			
	mean	412.19	18.55	17.27
	sd	67.49	2.05	3.77
	n	36	36	36
HighConc	2 / week			
	mean	427.34	18.05	16.87
	sd	52.26	1.82	3.40
	n	35	35	35
	4 / week			
	mean	439.89	17.42	19.75
	sd	43.98	2.22	6.95
	n	36	36	36
OVERALL	mean	428.23	17.96	18.03
	sd	54.45	2.03	5.14
	n	143	143	143

TABLE 3  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Duration Subgroups  
 (Averaging over Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	DURATION	ALP	ALT	BUN	CREA	TP	ALBG	CA	TBA	PHOS	SDH		
LowConc	1 Hr/Day												
		mean	435.72	41.69	17.76	.55	6.51	3.14	11.32	19.16	8.56	20.02	
		sd	49.19	3.93	1.79	.07	.24	.17	.33	5.03	1.59	5.02	
		n	36	36	36	36	36	36	36	36	36	36	
	4 Hr/Day												
		mean	409.94	45.97	18.63	.57	6.69	3.33	11.41	16.31	7.32	24.39	
		sd	66.93	8.75	2.14	.09	.22	.17	.38	3.67	1.04	8.24	
		n	36	36	36	36	36	36	36	36	36	36	
	HighConc	1 Hr/Day											
			mean	429.29	44.17	18.51	.58	6.83	3.38	11.61	17.99	7.35	23.10
			sd	49.70	5.38	1.72	.08	.27	.13	.39	5.52	1.29	5.73
			n	35	35	35	35	35	35	35	35	35	35
4 Hr/Day													
		mean	438.00	41.89	16.97	.54	6.52	3.14	11.31	18.66	8.34	19.44	
		sd	47.22	3.18	2.06	.06	.33	.17	.37	5.82	1.09	4.40	
		n	36	36	36	36	36	36	36	36	36	36	
OVERALL	mean	428.23	43.43	17.96	.56	6.63	3.24	11.41	18.03	7.90	21.73		
	sd	54.45	5.94	2.03	.08	.30	.19	.38	5.14	1.38	6.32		
	n	143	143	143	143	143	143	143	143	143	143		

TABLE 4  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	FREQUENCY	CK	ALP	ALT	CREA	TBA	SDH
Male	2 / week						
		mean	143.26	447.66	42.80	.53	15.65
		sd	44.30	49.71	3.01	.06	2.41
		n	35	35	35	35	35
	4 / week						
		mean	208.36	411.42	46.56	.60	19.25
		sd	122.21	70.93	7.02	.09	5.97
		n	36	36	36	36	36
Female	2 / week						
		mean	213.61	413.72	42.94	.59	19.40
		sd	120.91	46.28	7.46	.07	5.26
		n	36	36	36	36	36
	4 / week						
		mean	158.89	440.67	41.39	.52	17.77
		sd	63.05	37.58	3.79	.07	5.38
		n	36	36	36	36	36
OVERALL	mean	181.29	428.23	43.43	.56	18.03	21.73
	sd	98.33	54.45	5.94	.08	5.14	6.32
	n	143	143	143	143	143	143

TABLE 5  
L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
Means, SDs, and Ns for Sex by Duration Subgroups  
(Averaging over Concentration, Frequency, and Period)  
(variables with significant univariate F-test results)

SEX	DURATION	CK	GLU	TP	ALBG	TBA	PHOS		
Male	1 Hr/Day								
		mean	200.11	166.08	6.71	3.23	16.45	8.99	
		sd	123.62	32.71	.33	.21	3.66	1.16	
		n	35	35	35	35	35	35	
	4 Hr/Day								
		mean	153.08	147.43	6.80	3.34	18.46	8.00	
		sd	54.98	22.24	.14	.15	5.73	.74	
		n	36	36	36	36	36	36	
	Female	1 Hr/Day							
			mean	168.56	146.16	6.63	3.28	20.66	6.96
			sd	70.86	19.35	.27	.17	5.80	1.22
			n	36	36	36	36	36	36
4 Hr/Day									
		mean	203.94	148.57	6.41	3.12	16.51	7.67	
		sd	120.29	28.30	.28	.18	3.93	1.48	
		n	36	36	36	36	36	36	
OVERALL		mean	181.29	151.96	6.63	3.24	18.03	7.90	
		sd	98.33	27.09	.30	.19	5.14	1.38	
		n	143	143	143	143	143	143	

TABLE 6  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Concentration Subgroups  
 (Averaging over Frequency, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	CONCENTRATION	ALP	TP	ALBG	CA	PHOS
Male	LowConc					
		mean	416.19	6.62	3.24	11.32
		sd	74.80	.22	.21	.38
		n	36	36	36	36
	HighConc					
		mean	442.74	6.89	3.33	11.60
		sd	47.00	.21	.15	.38
		n	35	35	35	35
Female	LowConc					
		mean	429.47	6.58	3.22	11.41
		sd	39.45	.27	.18	.34
		n	36	36	36	36
	HighConc					
		mean	424.92	6.46	3.18	11.32
		sd	48.59	.31	.21	.38
		n	36	36	36	36
OVERALL		mean	428.23	6.63	3.24	11.41
		sd	54.45	.30	.19	.38
		n	143	143	143	143

TABLE 7  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period by Sex Subgroups  
 (Averaging over Concentration, Duration, and Frequency)  
 (variables with significant univariate F-test results)

PERIOD	SEX	ALBG	CHOL	TRIG
Post-Exp	Male			
		mean	3.33	45.95
		sd	.21	5.31
		n	36	36
	Female			
		mean	3.18	62.50
		sd	.19	5.66
		n	36	36
Post-Rec	Male			
		mean	3.23	47.38
		sd	.15	4.18
		n	35	35
	Female			
		mean	3.23	54.91
		sd	.19	5.15
		n	36	36
OVERALL	mean	3.24	52.72	120.35
	sd	.19	8.35	29.61
	n	143	143	143

TABLE 8  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Period)  
 (variables with significant univariate F-test results)

SEX		ALT	GLU	TP	ALBG	CHOL	TRIG	PHOS	SDH
Male									
	mean	44.70	156.62	6.75	3.28	46.65	134.90	8.49	19.74
	sd	5.71	29.24	.25	.19	4.81	34.00	1.09	6.96
	n	71	71	71	71	71	71	71	71
Female									
	mean	42.17	147.37	6.52	3.20	58.71	106.01	7.31	23.69
	sd	5.93	24.10	.29	.19	6.59	13.91	1.39	4.93
	n	72	72	72	72	72	72	72	72
OVERALL	mean	43.43	151.96	6.63	3.24	52.72	120.35	7.90	21.73
	sd	5.94	27.09	.30	.19	8.35	29.61	1.38	6.32
	n	143	143	143	143	143	143	143	143

TABLE 9  
 L06234 Clinical Chemistry Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroup:  
 (Averaging over Concentration, Duration, Frequency, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	ALP	BUN	CHOL	CA	TBA	PHOS
Post-Exp						
mean	439.07	17.41	54.23	11.27	15.78	7.72
sd	64.05	2.06	9.96	.37	3.68	1.52
n	72	72	72	72	72	72
Post-Rec						
mean	417.24	18.53	51.20	11.55	20.31	8.08
sd	40.13	1.85	6.01	.34	5.41	1.20
n	71	71	71	71	71	71
OVERALL						
mean	428.23	17.96	52.72	11.41	18.03	7.90
sd	54.45	2.03	8.35	.38	5.14	1.38
n	143	143	143	143	143	143



TABLE 10  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Period and Sex*  
(averaging over frequency and duration)

	concentration	significant increases	significant decreases
<i>Male Post-Exposure</i>	100 mg/m <sup>3</sup>		<b>2.</b>
	200 mg/m <sup>3</sup>		
	positive control		
<i>Male Post-Recovery</i>	100 mg/m <sup>3</sup>	9.	<b>3,7,11.</b>
	200 mg/m <sup>3</sup>	5,9,	<b>3.</b>
	positive control	5,	
<i>Female Post-Exposure</i>	100 mg/m <sup>3</sup>	<b>10.</b>	<b>2,13.</b>
	200 mg/m <sup>3</sup>	<b>10.</b>	<b>2.</b>
	positive control	10,	<b>2,11,13.</b>
<i>Female Post-Recovery</i>	100 mg/m <sup>3</sup>	<b>7,10,11.</b>	<b>3.</b>
	200 mg/m <sup>3</sup>	<b>10.</b>	<b>3.</b>
	positive control	7,10,	<b>3,9.</b>

*Key for significant effects*

1 = CK, 2 = ALP, 3 = ALT, 4 = BUN, 5 = CREA,  
6 = GLU, 7 = TP, 8 = ALBG, 9 = CHOL, 10 = TRIG,  
11 = CA, 12 = TBA, 13 = PHOS, 14 = SDH

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 11  
L06234 Clinical Chemistry Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	CK	ALP	ALT	BUN	CREA	GLU	TP	
Male	Post-Exp	Control	mean	167.08	485.58	46.63	17.54	.62	154.78	6.82
			sd	56.71	30.07	5.24	2.15	.23	24.53	.47
			n	24	24	24	24	24	24	24
			LowConc							
		mean	170.94	417.06	45.89	17.59	.59	142.76	6.63	
		sd	72.62	99.56	8.39	2.18	.11	22.74	.26	
		n	18	18	18	18	18	18	18	
		HighConc								
		mean	154.33	462.17	44.72	16.89	.58	164.47	6.93	
		sd	60.14	47.68	6.20	1.70	.09	33.62	.27	
		n	18	18	18	18	18	18	18	
		PosCont								
		mean	205.75	509.92	48.25	16.85	.58	148.92	6.83	
		sd	68.84	64.96	7.59	2.71	.09	27.95	.47	
		n	12	12	12	12	12	12	12	
		Post-Rec	Control	mean	213.42	432.00	50.88	19.37	.51	152.41
	sd			79.48	44.51	7.81	2.23	.07	23.22	.23
	n			24	24	24	24	24	24	24
	LowConc									
	mean		222.00	415.33	43.94	18.78	.53	157.97	6.61	
	sd		160.46	40.06	3.78	1.99	.06	23.10	.19	
	n		18	18	18	18	18	18	18	
	HighConc									
	mean		156.71	422.18	44.24	18.05	.56	161.57	6.84	
	sd		37.48	37.49	3.03	1.68	.05	33.33	.10	
	n		17	17	17	17	17	17	17	
	PosCont									
	mean		243.27	430.09	47.18	18.13	.57	137.01	6.69	
	sd		98.64	25.40	3.57	2.50	.05	19.10	.16	
	n		11	11	11	11	11	11	11	

TABLE 11  
L06234 Clinical Chemistry Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	CK	ALP	ALT	BUN	CREA	GLU	TP
Female	Post-Exp	Control							
		mean	175.54	490.83	43.46	18.15	.56	148.50	6.56
		sd	65.80	51.22	4.18	2.06	.08	14.29	.41
		n	24	24	24	24	24	24	24
		LowConc							
		mean	175.83	444.06	42.78	18.16	.57	147.22	6.52
		sd	79.02	39.44	10.33	1.67	.08	18.49	.29
		n	18	18	18	18	18	18	18
		HighConc							
		mean	181.33	433.00	41.89	16.99	.54	146.42	6.45
		sd	81.82	47.44	3.34	2.49	.09	26.14	.32
		n	18	18	18	18	18	18	18
	PosCont								
	mean	160.33	418.42	45.42	17.67	.58	140.50	6.26	
	sd	53.48	58.38	7.59	3.57	.08	27.23	.71	
	n	12	12	12	12	12	12	12	
	Post-Rec	Control							
		mean	175.29	431.29	47.42	19.50	.52	140.88	6.43
		sd	53.71	39.54	6.51	1.34	.06	13.79	.32
		n	24	24	24	24	24	24	24
		LowConc							
		mean	192.00	414.89	42.72	18.26	.56	147.37	6.63
		sd	84.94	34.61	3.23	2.13	.07	25.89	.24
		n	18	18	18	18	18	18	18
HighConc									
mean		195.83	416.83	41.28	18.99	.54	148.46	6.48	
sd		145.00	49.71	4.10	1.52	.06	26.98	.32	
n		18	18	18	18	18	18	18	
PosCont									
mean	219.92	426.75	41.75	18.30	.55	131.64	6.68		
sd	112.03	31.46	3.91	1.68	.06	8.33	.27		
n	12	12	12	12	12	12	12		

TABLE 11 (continued)  
L06234 Clinical Chemistry Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH
Male	Post-Exp	Control							
			mean	3.33	44.72	117.53	11.48	17.15	8.76
			sd	.29	4.23	28.85	.49	4.86	1.21
			n	24	24	24	24	24	24
		LowConc							
			mean	3.26	45.86	127.98	11.16	15.99	8.86
			sd	.23	6.86	42.67	.36	4.12	1.14
			n	18	18	18	18	18	18
		HighConc							
			mean	3.41	45.04	124.06	11.46	14.42	7.94
			sd	.15	3.31	34.73	.39	1.95	.94
			n	18	18	18	18	18	18
		PosCont							
			mean	3.35	49.93	141.46	11.54	15.88	8.37
			sd	.27	6.81	21.86	.51	5.03	.98
			n	12	12	12	12	12	12
	Post-Rec	Control							
			mean	3.29	44.53	132.82	11.76	21.22	8.77
			sd	.15	3.38	26.49	.23	6.14	.83
			n	24	24	24	24	24	24
		LowConc							
			mean	3.22	47.61	150.11	11.48	18.51	8.76
			sd	.20	4.96	27.83	.33	3.70	1.29
			n	18	18	18	18	18	18
		HighConc							
			mean	3.25	47.14	137.60	11.75	21.17	8.40
			sd	.09	3.28	23.75	.31	6.28	.72
			n	17	17	17	17	17	17
		PosCont							
			mean	3.20	43.91	135.75	11.69	19.33	8.45
			sd	.10	5.37	22.71	.20	5.13	1.51
			n	11	11	11	11	11	11

TABLE 11 (continued)  
 L06234 Clinical Chemistry Analysis of All Animals  
 Means, SDs, and Ns for Sex by Period Subgroups  
 (Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH
Female	Post-Exp	Control							
		mean	3.21	58.29	93.58	11.35	17.54	7.61	25.63
		sd	.25	4.70	7.62	.42	5.40	1.29	6.81
		n	24	24	24	24	24	24	24
		LowConc							
		mean	3.19	62.41	107.35	11.23	16.11	6.60	23.29
		sd	.19	5.57	16.15	.29	3.46	1.30	5.57
		n	18	18	18	18	18	18	18
		HighConc							
		mean	3.16	62.60	107.39	11.23	16.59	7.48	23.70
		sd	.19	5.91	11.69	.40	4.59	1.73	5.93
		n	18	18	18	18	18	18	18
	PosCont								
	mean	3.08	59.91	105.48	10.88	16.36	6.53	23.35	
	sd	.40	8.89	22.33	.54	3.38	1.34	4.35	
	n	12	12	12	12	12	12	12	
	Post-Rec	Control							
		mean	3.18	54.71	89.75	11.27	21.64	7.47	25.50
		sd	.20	5.92	9.71	.38	5.44	1.10	5.26
		n	24	24	24	24	24	24	24
		LowConc							
		mean	3.26	56.41	102.46	11.59	20.33	7.54	24.73
		sd	.16	4.71	13.11	.29	5.66	.88	4.40
		n	18	18	18	18	18	18	18
HighConc									
mean		3.21	53.42	106.84	11.41	21.29	7.63	23.03	
sd		.23	5.26	14.84	.35	5.70	1.38	3.75	
n		18	18	18	18	18	18	18	
PosCont									
mean	3.20	50.99	100.71	11.39	17.88	7.58	24.70		
sd	.19	5.75	9.43	.23	5.14	.76	3.37		
n	12	12	12	12	12	12	12		

TABLE 12  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Exposure Animals by Frequency and Duration*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	M		<b>7,8,14,</b>
100 mg/m <sup>3</sup>	2	4	F	5,10,	<b>2,13,</b>
100 mg/m <sup>3</sup>	4	1	F	10,	<b>14,</b>
100 mg/m <sup>3</sup>	4	4	M	14,	<b>2,</b>
200 mg/m <sup>3</sup>	2	1	F	10,	<b>2,13,</b>
200 mg/m <sup>3</sup>	2	4	M		1,3,13,14,
200 mg/m <sup>3</sup>	4	1	M	6,	
200 mg/m <sup>3</sup>	4	4	F	10,13,	5,7,8,11,14
positive control			M		
positive control			F	10,	<b>2,7,11,13,</b>

*Key for significant effects*

1 = CK, 2 = ALP, 3 = ALT, 4 = BUN, 5 = CREA,  
6 = GLU, 7 = TP, 8 = ALBG, 9 = CHOL, 10 = TRIG,  
11 = CA, 12 = TBA, 13 = PHOS, 14 = SDH

normal text:  $p < .05$ , **bold text:**  $p < .01$

TABLE 13  
L06234 Clinical Chemistry Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP		
Male	Control	4 / week	4 Hr/Day									
				mean	167.08	485.58	46.63	17.54	.62	154.78	6.82	
				sd	56.71	30.07	5.24	2.15	.23	24.53	.47	
				n	24	24	24	24	24	24	24	
	LowConc	2 / week	1 Hr/Day									
				mean	147.78	475.22	42.67	16.73	.54	147.58	6.43	
				sd	53.60	43.79	2.40	1.82	.10	24.89	.17	
				n	9	9	9	9	9	9	9	
		4 / week	4 Hr/Day									
				mean	194.11	358.89	49.11	18.46	.64	137.93	6.83	
				sd	84.41	107.39	10.98	2.27	.11	20.66	.15	
				n	9	9	9	9	9	9	9	
		HighConc	2 / week	4 Hr/Day								
					mean	120.22	468.11	40.78	16.58	.53	147.30	6.84
					sd	31.50	45.99	2.22	1.82	.05	12.89	.19
					n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day										
			mean	188.44	456.22	48.67	17.21	.64	181.64	7.02		
			sd	63.84	51.34	6.46	1.61	.09	39.65	.32		
			n	9	9	9	9	9	9	9		
	PosCont	4 / week	4 Hr/Day									
				mean	205.75	509.92	48.25	16.85	.58	148.92	6.83	
				sd	68.84	64.96	7.59	2.71	.09	27.95	.47	
				n	12	12	12	12	12	12	12	

TABLE 13  
L06234 Clinical Chemistry Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP		
Female	Control	4 / week	4 Hr/Day	mean	175.54	490.83	43.46	18.15	.56	148.50	6.56	
				sd	65.80	51.22	4.18	2.06	.08	14.29	.41	
				n	24	24	24	24	24	24	24	
		LowConc	2 / week	4 Hr/Day	mean	215.89	430.22	47.00	18.08	.62	153.63	6.58
					sd	96.05	44.49	13.32	1.60	.07	23.46	.29
					n	9	9	9	9	9	9	9
	4 / week		1 Hr/Day	mean	135.78	457.89	38.56	18.23	.52	140.81	6.46	
				sd	20.87	29.93	3.00	1.83	.05	9.16	.29	
				n	9	9	9	9	9	9	9	
		HighConc	2 / week	1 Hr/Day	mean	210.11	413.78	42.33	18.22	.59	151.01	6.71
					sd	91.23	48.43	3.74	2.06	.08	22.46	.21
					n	9	9	9	9	9	9	9
	4 / week		4 Hr/Day	mean	152.56	452.22	41.44	15.77	.50	141.83	6.19	
				sd	63.56	40.08	3.05	2.36	.08	30.01	.13	
				n	9	9	9	9	9	9	9	
		PosCont	4 / week	4 Hr/Day	mean	160.33	418.42	45.42	17.67	.58	140.50	6.26
					sd	53.48	58.38	7.59	3.57	.08	27.23	.71
					n	12	12	12	12	12	12	12



TABLE 13 (continued)  
 L06234 Clinical Chemistry Analysis of Post-Exposure Animals  
 Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH	
Male	Control	4 / week	4 Hr/Day	mean	3.33	44.72	117.53	11.48	17.15	8.76	18.77
				sd	.29	4.23	28.85	.49	4.86	1.21	3.62
				n	24	24	24	24	24	24	24
	LowConc	2 / week	1 Hr/Day	mean	3.09	47.86	144.79	11.09	14.88	9.53	15.24
				sd	.15	4.37	34.11	.11	3.06	1.18	3.59
				n	9	9	9	9	9	9	9
		4 / week	4 Hr/Day	mean	3.42	43.86	111.17	11.22	17.11	8.18	26.84
				sd	.16	8.49	45.50	.50	4.89	.57	13.94
				n	9	9	9	9	9	9	9
	HighConc	2 / week	4 Hr/Day	mean	3.36	45.58	117.10	11.43	14.41	7.84	14.96
				sd	.14	2.90	46.87	.24	1.53	1.02	1.46
				n	9	9	9	9	9	9	9
		4 / week	1 Hr/Day	mean	3.47	46.50	131.02	11.49	14.43	8.04	22.89
				sd	.15	3.78	16.03	.52	2.40	.90	6.63
				n	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day	mean	3.35	49.93	141.46	11.54	15.88	8.37	22.20
				sd	.27	6.81	21.86	.51	5.03	.98	6.51
				n	12	12	12	12	12	12	12

TABLE 13 (continued)  
L06234 Clinical Chemistry Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH		
Female	Control	4 / week	4 Hr/Day									
				mean	3.21	58.29	93.58	11.35	17.54	7.61	25.63	
				sd	.25	4.70	7.62	.2	5.40	1.29	6.81	
				n	24	24	24	24	24	24	24	
		LowConc	2 / week	4 Hr/Day								
					mean	3.21	62.33	107.92	11.28	15.76	6.44	26.89
					sd	.18	6.71	18.04	.35	4.72	1.39	4.02
					n	9	9	9	9	9	9	9
			4 / week	1 Hr/Day								
	mean				3.18	62.48	106.78	11.19	16.47	6.76	19.69	
	sd				.21	4.58	15.10	.23	1.71	1.27	4.53	
	n				9	9	9	9	9	9	9	
	HighConc			2 / week	1 Hr/Day							
		mean				3.33	62.62	106.99	11.56	17.46	6.09	26.49
		sd				.07	4.41	8.40	.25	4.71	.95	5.39
		n				9	9	9	9	9	9	9
		4 / week		4 Hr/Day								
			mean		2.98	62.58	107.80	10.90	15.73	8.87	20.91	
			sd		.07	7.39	14.82	.17	4.58	1.07	5.30	
			n		9	9	9	9	9	9	9	
			PosCont	4 / week	4 Hr/Day							
	mean					3.08	59.91	105.48	10.88	16.36	6.53	23.35
	sd					.40	8.89	22.33	.54	3.38	1.34	4.35
	n					12	12	12	12	12	12	12

TABLE 14  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Recovery Animals by Duration and Frequency*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	F	7,10,11,	3,4,
100 mg/m <sup>3</sup>	2	4	M	8,9,10,	1,3,12,13,
100 mg/m <sup>3</sup>	4	1	M	13,	3,7,8,11,
100 mg/m <sup>3</sup>	4	4	F	10,11,	12,
200 mg/m <sup>3</sup>	2	1	M	10,	3,12,
200 mg/m <sup>3</sup>	2	4	F	10,13,	3,8,
200 mg/m <sup>3</sup>	4	1	F	7,8,10,11,	3,
200 mg/m <sup>3</sup>	4	4	M	5,9,12,	3,8,
positive control			M	5,	3,8,
positive control			F	7,10,	3,4,12,

*Key for significant effects*

1 = CK, 2 = ALP, 3 = ALT, 4 = BUN, 5 = CREA.  
6 = GLU, 7 = TP, 8 = ALBG, 9 = CHOL, 10 = TRIG,  
11 = CA, 12 = TBA, 13 = PHOS, 14 = SDH

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 15  
L06234 Clinical Chemistry Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP				
Male	Control	4 / week	4 Hr/Day	mean	213.42	432.00	50.88	19.37	.51	152.41	6.75			
				sd	79.48	44.51	7.81	2.23	.07	23.22	.23			
				n	24	24	24	24	24	24	24			
				LowConc	2 / week	4 Hr/Day	mean	145.56	424.67	43.89	19.02	.52	147.72	6.72
							sd	36.66	42.88	3.18	2.27	.07	16.19	.11
							n	9	9	9	9	9	9	9
		4 / week	1 Hr/Day				mean	298.44	406.00	44.00	18.53	.54	168.22	6.50
							sd	200.56	37.08	4.50	1.77	.06	25.21	.19
							n	9	9	9	9	9	9	9
				HighConc	2 / week	1 Hr/Day	mean	161.50	419.50	44.00	19.09	.53	166.99	6.89
							sd	49.84	46.81	3.42	.68	.04	34.73	.10
							n	8	8	8	8	8	8	8
		4 / week	4 Hr/Day				mean	152.44	424.56	44.44	17.12	.59	156.76	6.79
							sd	24.36	29.65	2.83	1.78	.04	33.32	.08
							n	9	9	9	9	9	9	9
				PosCont	4 / week	4 Hr/Day	mean	243.27	430.09	47.18	18.13	.57	137.01	6.69
							sd	98.64	25.40	3.57	2.50	.05	19.10	.16
							n	11	11	11	11	11	11	11

TABLE 15  
L06234 Clinical Chemistry Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP		
Female	Control	4 / week	4 Hr/Day									
				mean	175.29	431.29	47.42	19.50	.52	140.88	6.43	
				sd	53.71	39.54	6.51	1.34	.06	13.79	.32	
				n	24	24	24	24	24	24	24	
	LowConc	2 / week	1 Hr/Day									
				mean	182.56	403.78	41.56	17.54	.60	144.64	6.66	
				sd	84.70	44.13	3.78	1.41	.06	14.89	.25	
				n	9	9	9	9	9	9	9	
		4 / week	4 Hr/Day									
				mean	201.44	426.00	43.89	18.97	.52	150.10	6.61	
				sd	89.21	17.89	2.20	2.55	.07	34.44	.23	
				n	9	9	9	9	9	9	9	
		HighConc	2 / week	4 Hr/Day								
					mean	245.89	407.11	40.89	18.42	.54	148.72	6.26
					sd	192.25	51.29	3.44	1.54	.05	28.01	.17
					n	9	9	9	9	9	9	9
	4 / week	1 Hr/Day										
			mean	145.78	426.56	41.67	19.57	.54	148.19	6.70		
			sd	45.63	49.06	4.85	1.34	.08	27.61	.27		
			n	9	9	9	9	9	9	9		
	PosCont	4 / week	4 Hr/Day									
				mean	219.92	426.75	41.75	18.30	.55	131.64	6.68	
				sd	112.03	31.46	3.91	1.68	.06	8.33	.27	
				n	12	12	12	12	12	12	12	

TABLE 15  
L06234 Clinical Chemistry Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	CK	ALP	ALT	BUN	CREA	GLU	TP	
Male	Control	4 / week	4 Hr/Day	mean	213.42	432.00	50.88	19.37	.51	152.41	6.75
				sd	79.48	44.51	7.81	2.23	.07	23.22	.23
				n	24	24	24	24	24	24	24
	LowConc	2 / week	4 Hr/Day	mean	145.56	424.67	43.89	19.02	.52	147.72	6.72
				sd	36.66	42.88	3.18	2.27	.07	16.19	.11
				n	9	9	9	9	9	9	9
		4 / week	1 Hr/Day	mean	298.44	406.00	44.00	18.53	.54	168.22	6.50
				sd	200.56	37.08	4.50	1.77	.06	25.21	.19
				n	9	9	9	9	9	9	9
	HighConc	2 / week	1 Hr/Day	mean	161.50	419.50	44.00	19.09	.53	166.99	6.89
				sd	49.84	46.81	3.42	.68	.04	34.73	.10
				n	8	8	8	8	8	8	8
	4 / week	4 Hr/Day	mean	152.44	424.56	44.44	17.12	.59	156.76	6.79	
			sd	24.36	29.65	2.83	1.78	.04	33.32	.08	
			n	9	9	9	9	9	9	9	
PosCont	4 / week	4 Hr/Day	mean	243.27	430.09	47.18	18.13	.57	137.01	6.69	
			sd	98.64	25.40	3.57	2.50	.05	19.10	.16	
			n	11	11	11	11	11	11	11	

TABLE 15 (continued)  
L06234 Clinical Chemistry Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH	
Male	Control	4 / week	4 Hr/Day	mean	3.29	44.53	132.82	11.76	21.22	8.77	20.93
				sd	.15	3.38	26.49	.23	6.14	.83	5.93
				n	24	24	24	24	24	24	24
	LowConc	2 / week	4 Hr/Day	mean	3.39	49.26	166.26	11.56	16.94	7.71	18.07
				sd	.07	5.20	24.01	.20	2.46	.50	2.76
				n	9	9	9	9	9	9	9
		4 / week	1 Hr/Day	mean	3.06	45.97	133.97	11.41	20.07	9.80	21.47
				sd	.11	4.39	21.97	.42	4.19	.91	3.60
				n	9	9	9	9	9	9	9
	HighConc	2 / week	1 Hr/Day	mean	3.31	46.38	151.85	11.89	16.44	8.55	18.05
				sd	.06	4.29	21.65	.36	1.54	.72	2.39
				n	8	8	8	8	8	8	8
		4 / week	4 Hr/Day	mean	3.19	47.82	124.93	11.62	25.38	8.27	20.24
				sd	.06	2.08	18.29	.20	5.88	.75	4.32
				n	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day	mean	3.20	43.91	135.75	11.69	19.33	8.45	22.87
				sd	.10	5.37	22.71	.20	5.13	1.51	6.94
				n	11	11	11	11	11	11	11

TABLE 15 (continued)  
L06234 Clinical Chemistry Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	ALBG	CHOL	TRIG	CA	TBA	PHOS	SDH	
Female	Control	4 / week	4 Hr/Day	mean	3.18	54.71	89.75	11.27	21.64	7.47	25.50
				sd	.20	5.92	9.71	.38	5.44	1.10	5.26
				n	24	24	24	24	24	24	24
	LowConc	2 / week	1 Hr/Day	mean	3.22	56.68	102.43	11.60	25.24	8.13	23.69
				sd	.14	4.14	13.64	.25	3.05	.79	4.53
				n	9	9	9	9	9	9	9
		4 / week	4 Hr/Day	mean	3.29	56.13	102.48	11.58	15.42	6.96	25.78
				sd	.18	5.47	13.39	.34	2.10	.47	4.26
				n	9	9	9	9	9	9	9
	HighConc	2 / week	4 Hr/Day	mean	3.02	52.87	112.01	11.28	19.13	8.40	21.64
				sd	.07	4.87	17.01	.38	3.06	1.36	2.10
				n	9	9	9	9	9	9	9
		4 / week	1 Hr/Day	mean	3.40	53.98	101.68	11.53	23.46	6.87	24.42
				sd	.16	5.85	10.87	.27	7.01	.93	4.60
				n	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day	mean	3.20	50.99	100.71	11.39	17.88	7.58	24.70
				sd	.19	5.75	9.43	.23	5.14	.76	3.37
				n	12	12	12	12	12	12	12



PART TWO

APPENDIX

SECTION E. HEMATOLOGY

HIT RESEARCH INSTITUTE

SA/E-1

# 1 L06234 Statistical Report - Hematology

Multivariate analysis of variance models (Bock, 1975) were used to analyze the hematology variables: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT. Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: Sex (male or female), Period (exposure or recovery), concentration (control, 100  $mg/m^3$ , 200  $mg/m^3$ , or positive control at 200  $mg/m^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using a multivariate analysis of variance model which included all five factors and all two way interactions. The second set of analyses concentrated on examining the differences between the dosed and control animals. These analyses were performed separately in each of the four subsamples, defined by period and sex, in order to examine group differences while controlling for the effects of period and sex. First, a one-factor multivariate analysis of variance was performed on the eight hematology variables, with concentration as the grouping factor, augmented by simple contrasts which allowed a statistical comparison to be made between each group (100  $mg/m^3$ , 200  $mg/m^3$ , and positive control at 200  $mg/m^3$ ) and the control group. In order to examine the consistency of any group differences between the levels of frequency and duration, a second multivariate analysis of variance was performed. In this second analysis, the low and high dose animals were further grouped depending on their duration and frequency of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration and frequency subgroups.

## 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance yielded significant interaction effects of sex by frequency ( $p < .001$ ), concentration by duration ( $p < .001$ ), period by frequency ( $p < .006$ ), and sex by period ( $p < .001$ ) by the multivariate test. Significant main effects of sex ( $p < .001$ ) and period ( $p < .001$ ) were also observed. All other main effects and two way interactions were observed to be non-significant by the multivariate test.

The sex by frequency interaction was observed to be significant for RBC, HCT, MCH, MCHC ( $p < .001$  for all), and PLT ( $p < .003$ ) by the univariate F-tests. The observed means for the subgroups defined by sex and frequency, listed in Table 1, illustrate the nature of the sex by frequency interaction for these variables. For males, the frequency of 4 per week elevated RBC and HCT values and decreased MCH, MCHC, and PLT values, relative to 2 exposures per week, while for the females the reverse was observed: RBC and HCT values were decreased with the 4 per week frequency and MCH, MCHC, and PLT were increased by the 4 per week frequency, relative to the 2 per week frequency.

Regarding the concentration by duration interaction, significant effects were observed for MCV ( $p < .001$ ) and MCHC ( $p < .001$ ) by the subsequent univariate F-tests. The observed means for the subgroups defined by concentration and duration are given in Table 2. These observed means indicate that the low dosed animals had elevated MCV values when exposed for 1 hour per day and decreased MCV values when exposed for 4 hours per day, relative to the high dosed animals. For MCHC this interaction is reversed, in that, the low dosed animals had decreased MCHC values

when exposed for 1 hour per day and increased MCHC values when exposed for 4 hours per day, relative to the high dosed animals.

The period by frequency interaction was observed to be significant for RBC ( $p < .002$ ), HCT ( $p < .024$ ), MCV ( $p < .001$ ), and MCH ( $p < .003$ ) by the univariate F-tests. The observed means for the subgroups defined by period and frequency, listed in Table 3, illustrate the nature of the period by frequency interaction for these variables. For the exposed animals, the frequency of 4 per week elevated RBC and HCT values and decreased MCV and MCH values, relative to 2 exposures per week, while for the recovered animals the reverse was observed: RBC and HCT values were decreased with the 4 per week frequency and MCV and MCH were increased by the 4 per week frequency, relative to the 2 per week frequency.

The sex by period interaction was observed to be significant for WBC ( $p < .043$ ), MCV, MCH ( $p < .001$  for both), and PLT ( $p < .017$ ) by the univariate F-tests. The observed means for the subgroups defined by sex and period, listed in Table 4, illustrate the nature of the sex by period interaction for these variables. Male recovered animals had decreased MCH means, relative to the exposure animals, while female recovered animals had approximately the same mean as the female exposed animals. For the remaining variables, the direction of the exposure vs. recovery difference was the same, however the magnitude of the difference was not. For males, recovery animals had a much more dramatic increase over the exposure animals in WBC, than was the case in the females. Conversely for MCV, male recovered animals had a much more dramatic decrease compared with the male exposed animals, than was observed for the females. Finally, for females, recovery animals had a much more dramatic increase over the exposure animals in PLT, than was the case in the males.

The main effect of sex was observed to be statistically significant by the univariate test for the variables: RBC, HCT, MCV, MCH, and MCHC ( $p < .001$  for all, except  $p < .01$  for HCT). Table 5 which lists the observed means for these variables by sex indicates that males had elevated RBC and HCT means, and decreased MCV, MCH, and MCHC means, relative to females.

Finally, the main effect of period was observed to be statistically significant by the univariate test for the following hematology parameters: WBC, RBC, HGB, HCT, MCV, MCH, and PLT ( $p < .001$  for all, except  $p < .002$  for MCH). Table 6 which lists the observed means for these variables by period indicates that the exposure animals had elevated means on RBC, HGB, HCT, MCV, and MCH, and decreased WBC and PLT means, relative to the recovery animals.

## 1.2 Analysis of All Animals - Comparison to Controls

In the first set of analyses, concentration differences within each of the four subsamples defined by period and sex were examined. The significant differences that were observed are listed in Table 7, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 8.

The univariate tests performed on the male exposure animals revealed significant group differences only in terms of WBC ( $p < .016$ ). As Tables 7 and 8 reveal, the positive control group exhibited a significantly elevated mean on WBC, relative to the control group. For the female exposure animals, significant group differences were observed on WBC ( $p < .029$ ), MCV ( $p < .003$ ), and PLT ( $p < .006$ ). As indicated in Tables 7 and 8, all groups exhibited significant decreases on MCV and PLT as compared to controls. Also, the positive control group was significantly elevated in terms of WBC, as compared to the control group.

Turning to the results from the recovery animals, significant group differences for the male recovery animals were observed by the univariate tests on WBC ( $p < .001$ ), RBC ( $p < .016$ ), HGB ( $p < .014$ ), HCT ( $p < .015$ ), and PLT ( $p < .001$ ). Tables 7 and 8 indicate that the positive

control group was significantly elevated on each of these variables, relative to the control group. Additionally, both low and high dose groups were significantly elevated on PLT, while the low dose group was also significantly elevated on WBC, as compared to the controls. For the female recovery animals, significant group differences were observed on WBC ( $p < .004$ ) and PLT ( $p < .001$ ). As indicated in Tables 7 and 8, all groups exhibited significant increases on PLT, while the positive control group was also significantly elevated in terms of WBC, as compared to the control group.

In the second set of analyses, concentration differences were again examined within each of the four subsamples defined by period and sex. However, in order to also examine the effects of frequency and duration, the low and high dose animals were further divided into subgroups depending on their level of frequency and duration. The significant differences that were observed for the exposure animals are listed in Table 9, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 10. Similarly, the significant differences that were observed for the recovery animals are listed in Table 11, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 12.

The univariate tests performed on the male exposure animals revealed significant group differences in terms of WBC ( $p < .050$ ), RBC ( $p < .010$ ), HGB ( $p < .019$ ), HCT ( $p < .021$ ), and MCV ( $p < .047$ ). As Tables 9 and 10 reveal, the positive control group exhibited a significantly elevated mean on WBC, relative to the control group, while the low dose group with 4 weekly exposures of 4 hours per day exhibited elevated means on RBC, HGB, and HCT, relative to the control group. None of the comparisons with the control group were significant for MCV. For the female exposure animals, significant group differences were observed on MCV ( $p < .008$ ) and PLT ( $p < .003$ ). As indicated in Tables 9 and 10, all subgroups, with the exception of the low dose females with 4 weekly exposures of 1 hour per day, exhibited significant decreases on MCV as compared to controls. Also, for PLT, the positive control group and the low and high dose groups exposed for 4 hours per day had significantly decreased means, in contrast to the control group.

Significant group differences on the male recovery animals were observed by the univariate tests on WBC ( $p < .001$ ), RBC ( $p < .043$ ), HGB ( $p < .034$ ), HCT ( $p < .045$ ), MCV ( $p < .001$ ), MCHC ( $p < .036$ ), and PLT ( $p < .002$ ). Tables 11 and 12 indicate that the positive control group were significantly elevated on many of these variables (WBC, RBC, HGB, HCT, and PLT), relative to the control group. Additionally, all low and high dose subgroups, except the low dose males exposed 4 times weekly for 1 hour per day, were significantly elevated on PLT, while both subgroups exposed 4 hours per day were also significantly elevated on WBC, as compared to the controls. Both subgroups with 2 exposures per week had significantly decreased MCV means, while the subgroup with 4 weekly exposures of 4 hours per day had a significantly decreased MCHC mean, relative to the control group. For the female recovery animals, significant group differences were observed on WBC ( $p < .015$ ), RBC ( $p < .015$ ), HCT ( $p < .003$ ), MCH ( $p < .008$ ), MCHC ( $p < .001$ ) and PLT ( $p < .001$ ). As indicated in Tables 11 and 12, all groups exhibited significant increases on PLT, the positive control group was significantly elevated in terms of WBC, the high dose subgroup with 4 weekly exposures of 1 hour per day exhibited elevated MCHC values, and the low dose group with 2 weekly exposures of 1 hour per day exhibited increased RBC and HCT values, as compared to the control group. Significant decreases, as compared to the control group, were observed for the high dose group with 4 weekly exposures of 1 hour per day on HCT, and for the high dose group with 2 weekly exposures of 4 hours per day on MCH and MCHC.

### 1.3 Summary

In general, duration and frequency did not play a role in the effect of exposure to the compound. Male animals did not appear to exhibit any major exposure related effects at the treatment sacrifice.

although WBC and PLT levels were increased relative to controls following the recovery period. Female animals exhibited consistent decreases in MCV and PLT levels at the treatment sacrifice for both concentrations, but PLT values were increased relative to controls following the recovery period. When the differential effects of duration and frequency were examined, low dose male animals exposed for 4 hours 4 times per week did exhibit increases in RBC, HGB, and HCT, but this effect was not reproduced at the high dose or following the recovery period. Following the recovery period several significant increases (RBC, HCT, MCHC, and PLT) were observed for female animals at various concentrations, durations and frequencies. The most consistent effect was for PLT. In addition, high dose female animals did exhibit significant decreases in HCT, MCH and MCHC following the recovery period, but these differences were not consistent for any particular frequency or duration.

In summary, exposure to this compound produced consistent decreases in MCV and PLT for female animals regardless of concentration, duration, and frequency. Male animals were relatively unaffected. Following the recovery period the picture was somewhat more complex with both male and female animals exhibiting increased PLT levels, although a scatter of significant increases and decreases for several different outcome measures were observed when the data were broken down by duration and frequency. However, no consistent duration or frequency related effects were observed.

## References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.

TABLE 1  
 L06234 Hematology Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	FREQUENCY	RBC	HCT	MCH	MCHC	PLT		
Male	2 / week	mean	8.64	43.98	18.69	36.72	812.70	
		sd	.29	2.01	.47	.74	48.44	
		n	33	33	33	33	33	
	4 / week	mean	8.78	44.97	18.61	36.30	785.47	
		sd	.58	3.14	.45	.79	56.07	
		n	36	36	36	36	36	
	Female	2 / week	mean	8.49	44.35	19.19	36.76	789.72
			sd	.37	1.83	.51	.87	73.17
			n	36	36	36	36	36
4 / week		mean	8.23	43.04	19.57	37.43	816.61	
		sd	.41	2.36	.39	.89	74.26	
		n	36	36	36	36	36	
OVERALL		mean	8.53	44.09	19.02	36.80	800.88	
		sd	.47	2.47	.60	.91	65.03	
		n	141	141	141	141	141	

TABLE 2  
 L06234 Hematology Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Duration Subgroups  
 (Averaging over Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	DURATION	MCV	MCHC
LowConc	1 Hr/Day		
		mean	52.01
		sd	.84
		n	34
	4 Hr/Day		
		mean	51.44
		sd	1.15
		n	36
HighConc	1 Hr/Day		
		mean	51.43
		sd	.97
		n	35
	4 Hr/Day		
		mean	51.86
		sd	.70
		n	36
OVERALL	mean	51.68	36.80
		sd	.96
		n	141

TABLE 3  
 L06234 Hematology Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	FREQUENCY	RBC	HCT	MCV	MCH
Post-Exp	2 / week				
		mean	8.69	45.37	52.19
		sd	.33	1.62	.46
		n	34	34	34
	4 / week				
		mean	8.81	45.85	52.07
		sd	.55	2.62	.74
		n	36	36	36
Post-Rec	2 / week				
		mean	8.44	43.01	51.00
		sd	.29	1.40	1.24
		n	35	35	35
	4 / week				
		mean	8.20	42.17	51.47
		sd	.41	1.86	.69
		n	36	36	36
OVERALL		mean	8.53	44.09	51.68
		sd	.47	2.47	.96
		n	141	141	141



TABLE 4  
L06234 Hematology Analysis of Graphite-Exposed Animals  
Means, SDs, and Ns for Period by Sex Subgroups  
(Averaging over Concentration, Duration, and Frequency)  
(variables with significant univariate F-test results)

PERIOD	SEX	WBC	MCV	MCH	PLT
Post-Exp	Male				
	mean	9.61	51.77	18.87	774.03
	sd	1.85	.54	.43	52.53
	n	34	34	34	34
	Female				
	mean	9.93	52.47	19.37	757.31
	sd	2.01	.49	.34	62.45
	n	36	36	36	36
	Post-Rec	Male			
mean		11.57	50.41	18.43	822.26
sd		1.92	.72	.39	44.29
n		35	35	35	35
Female					
mean		10.48	52.04	19.39	849.03
sd		1.33	.47	.61	54.89
n		36	36	36	36
OVERALL		mean	10.40	51.68	19.02
	sd	1.93	.96	.60	65.03
	n	141	141	141	141

TABLE 5  
 L06234 Hematology Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Period)  
 (variables with significant univariate F-test results)

SEX	RBC	HCT	MCV	MCH	MCHC
Male					
mean	8.71	44.50	51.08	18.65	36.50
sd	.47	2.69	.93	.46	.79
n	69	69	69	69	69
Female					
mean	8.36	43.70	52.26	19.38	37.09
sd	.41	2.20	.53	.49	.94
n	72	72	72	72	72
OVERALL mean	8.53	44.09	51.68	19.02	36.80
sd	.47	2.47	.96	.60	.91
n	141	141	141	141	141

TABLE 6  
 L06234 Hematology Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	WBC	RBC	HGB	HCT	MCV	MCH	PLT
Post-Exp							
mean	9.77	8.75	16.73	45.62	52.13	19.13	765.43
sd	1.92	.46	.69	2.19	.62	.46	58.04
n	70	70	70	70	70	70	70
Post-Rec							
mean	11.02	8.31	15.70	42.58	51.24	18.92	835.83
sd	1.73	.37	.44	1.69	1.02	.70	51.39
n	71	71	71	71	71	71	71
OVERALL mean	10.40	8.53	16.21	44.09	51.68	19.02	800.88
sd	1.93	.47	.77	2.47	.96	.60	65.03
n	141	141	141	141	141	141	141

TABLE 7  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Period and Sex*  
(averaging over frequency and duration)

	concentration	significant increases	significant decreases
<i>Male Post-Exposure</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>		
	positive control	1	
<i>Male Post-Recovery</i>	100 mg/m <sup>3</sup>	1,8	
	200 mg/m <sup>3</sup>	8	
	positive control	1,2,3,4,8	
<i>Female Post-Exposure</i>	100 mg/m <sup>3</sup>		5,8
	200 mg/m <sup>3</sup>		5,8
	positive control	1	5,8
<i>Female Post-Recovery</i>	100 mg/m <sup>3</sup>	8	
	200 mg/m <sup>3</sup>	8	
	positive control	1,8	

*Key for significant effects*

1 = WBC, 2 = RBC, 3 = HGB, 4 = HCT,  
5 = MCV, 6 = MCH, 7 = MCHC, 8 = PLT  
normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 8  
L06234 Hematology Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Male	Post-Exp	Control								
		mean	9.10	8.58	16.33	44.55	51.90	19.04	36.68	794.00
		sd	2.15	.40	.66	2.07	.45	.42	.75	45.37
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	9.62	9.02	16.99	46.69	51.76	18.85	36.42	765.94
		sd	1.60	.71	1.16	3.59	.55	.44	.69	58.42
		n	16	16	16	16	16	16	16	16
		HighConc								
		mean	9.59	8.81	16.63	45.61	51.78	18.88	36.47	781.22
		sd	2.10	.26	.42	1.23	.55	.44	.71	47.21
		n	18	18	18	18	18	18	18	18
	PosCont									
	mean	11.33	8.73	16.52	45.49	52.17	18.95	36.33	787.25	
	sd	1.53	.56	.91	2.61	.86	.33	.58	82.17	
	n	12	12	12	12	12	12	12	12	
	Post-Rec	Control								
		mean	10.43	8.47	15.71	42.85	50.60	18.56	36.70	781.79
		sd	1.49	.33	.35	1.69	.65	.51	1.08	34.67
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	11.75	8.46	15.64	42.56	50.32	18.49	36.77	808.83
		sd	2.09	.29	.45	1.51	.81	.36	.77	48.66
		n	18	18	18	18	18	18	18	18
HighConc										
mean		11.38	8.57	15.72	43.30	50.50	18.37	36.34	836.47	
sd		1.77	.30	.46	1.63	.62	.41	.96	35.14	
n		17	17	17	17	17	17	17	17	
PosCont										
mean	13.42	8.81	16.15	44.55	50.57	18.34	36.27	820.27		
sd	2.35	.27	.45	1.64	.75	.36	.79	38.54		
n	11	11	11	11	11	11	11	11		

TABLE 8  
L06234 Hematology Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Female	Post-Exp	Control								
		mean	9.75	8.42	16.47	44.75	53.15	19.56	36.80	799.33
		sd	2.01	.36	.52	1.61	.79	.56	.78	50.42
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	10.07	8.60	16.69	45.21	52.55	19.42	36.96	756.67
		sd	1.81	.38	.56	1.86	.48	.37	.67	63.15
		n	18	18	18	18	18	18	18	18
		HighConc								
		mean	9.80	8.61	16.63	45.08	52.39	19.33	36.89	757.94
		sd	2.23	.27	.40	1.23	.50	.32	.60	63.55
		n	18	18	18	18	18	18	18	18
	PosCont									
	mean	11.94	8.06	15.57	42.30	52.62	19.37	36.84	725.67	
	sd	1.96	1.62	3.01	8.25	.88	.62	1.10	79.81	
	n	12	12	12	12	12	12	12	12	
	Post-Rec	Control								
		mean	10.37	8.08	15.71	42.18	52.20	19.47	37.29	769.96
		sd	1.58	.42	.51	1.99	.62	.68	1.19	48.91
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	10.39	8.17	15.84	42.68	52.23	19.41	37.13	857.50
		sd	1.48	.31	.44	1.62	.43	.52	.90	57.29
		n	18	18	18	18	18	18	18	18
HighConc										
mean		10.57	8.07	15.62	41.82	51.85	19.38	37.39	840.56	
sd		1.21	.36	.42	1.81	.44	.69	1.38	52.62	
n		18	18	18	18	18	18	18	18	
PosCont										
mean	12.62	8.20	15.87	42.82	52.23	19.37	37.07	834.33		
sd	2.24	.29	.45	1.65	.51	.57	1.28	59.34		
n	12	12	12	12	12	12	12	12		

TABLE 9  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Exposure Animals by Duration and Frequency*

concentration	duration (hr/day)	frequency (exp/week)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	1	2	M		
100 mg/m <sup>3</sup>	1	4	F		
100 mg/m <sup>3</sup>	4	2	F		5,8
100 mg/m <sup>3</sup>	4	4	M	<b>2,3,4</b>	
200 mg/m <sup>3</sup>	1	2	F		5
200 mg/m <sup>3</sup>	1	4	M		
200 mg/m <sup>3</sup>	4	2	M		
200 mg/m <sup>3</sup>	4	4	F		5,8
positive control			M	1	
positive control			F		5,8

*Key for significant effects*

1 = WBC, 2 = RBC, 3 = HGB, 4 = HCT,  
5 = MCV, 6 = MCH, 7 = MCHC, 8 = PLT  
normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 10  
L06234 Hematology Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	
Male	Control	4 / week	4 Hr/Day									
				mean	9.10	8.58	16.33	44.55	51.90	19.04	36.68	794.00
				sd	2.15	.40	.66	2.07	.45	.42	.75	45.37
				n	24	24	24	24	24	24	24	
	LowConc	2 / week	1 Hr/Day									
				mean	9.54	8.63	16.36	44.90	52.04	18.99	36.47	779.86
				sd	1.50	.42	.49	2.02	.56	.48	.67	54.55
				n	7	7	7	7	7	7	7	
		4 / week	4 Hr/Day									
				mean	9.68	9.33	17.48	48.08	51.53	18.74	36.38	755.11
				sd	1.75	.75	1.32	4.02	.45	.39	.73	62.18
				n	9	9	9	9	9	9	9	
		HighConc	2 / week	4 Hr/Day								
	mean				10.10	8.79	16.66	45.77	52.06	18.94	36.39	804.44
	sd				2.56	.19	.41	1.07	.44	.42	.73	45.47
				n	9	9	9	9	9	9	9	
	4 / week		1 Hr/Day									
				mean	9.09	8.83	16.61	45.46	51.51	18.82	36.54	758.00
sd				1.49	.32	.45	1.42	.52	.47	.73	38.15	
			n	9	9	9	9	9	9	9		
PosCont	4 / week		4 Hr/Day									
		mean		11.33	8.73	16.52	45.49	52.17	18.95	36.33	787.25	
		sd		1.53	.56	.91	2.61	.86	.33	.58	82.17	
		n	12	12	12	12	12	12	12			



TABLE 10  
L06234 Hematology Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Female	Control	4 / week	4 Hr/Day								
				mean	9.75	8.42	16.47	44.75	53.15	19.56	799.33
				sd	2.01	.36	.52	1.61	.79	.56	50.42
				n	24	24	24	24	24	24	24
	LowConc	2 / week	4 Hr/Day								
				mean	9.81	8.61	16.73	45.06	52.34	19.46	723.33
				sd	1.98	.43	.55	2.08	.43	.43	41.75
				n	9	9	9	9	9	9	9
		4 / week	1 Hr/Day								
				mean	10.32	8.60	16.66	45.36	52.76	19.38	790.00
				sd	1.70	.34	.59	1.72	.46	.32	65.06
				n	9	9	9	9	9	9	9
	HighConc	2 / week	1 Hr/Day								
				mean	9.26	8.73	16.79	45.66	52.30	19.24	770.00
				sd	2.13	.29	.41	1.31	.42	.36	74.77
				n	9	9	9	9	9	9	9
		4 / week	4 Hr/Day								
				mean	10.34	8.48	16.47	44.51	52.49	19.41	745.89
				sd	2.31	.21	.34	.89	.58	.26	51.63
				n	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day								
				mean	11.94	8.06	15.57	42.30	52.62	19.37	725.67
				sd	1.96	1.62	3.01	8.25	.88	.62	79.81
				n	12	12	12	12	12	12	12

TABLE 11  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Recovery Animals by Duration and Frequency*

concentration	duration (hr/day)	frequency (exp/week)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	1	2	F	2,4,8	
100 mg/m <sup>3</sup>	1	4	M		
100 mg/m <sup>3</sup>	4	2	M	1,8	5
100 mg/m <sup>3</sup>	4	4	F	8	
200 mg/m <sup>3</sup>	1	2	M	8	5
200 mg/m <sup>3</sup>	1	4	F	7,8	4
200 mg/m <sup>3</sup>	4	2	F	8	6,7
200 mg/m <sup>3</sup>	4	4	M	1,8	7
positive control			M	1,2,3,4,8	
positive control			F	1,8	

*Key for significant effects*

1 = WBC, 2 = RBC, 3 = HGB, 4 = HCT,  
5 = MCV, 6 = MCH, 7 = MCHC, 8 = PLT  
normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 12  
L06234 Hematology Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	
Male	Control	4 / week	4 Hr/Day									
				mean	10.43	8.47	15.71	42.85	50.60	18.56	36.70	781.79
				sd	1.49	.33	.35	1.69	.65	.51	1.08	34.67
				n	24	24	24	24	24	24	24	24
	LowConc	2 / week	4 Hr/Day									
				mean	12.78	8.54	15.72	42.43	49.69	18.40	37.07	816.22
				sd	1.60	.21	.38	1.13	.45	.33	.69	30.46
				n	9	9	9	9	9	9	9	9
		4 / week	1 Hr/Day									
				mean	10.72	8.38	15.56	42.69	50.94	18.59	36.47	801.44
				sd	2.08	.35	.51	1.87	.55	.38	.76	63.09
				n	9	9	9	9	9	9	9	9
	HighConc	2 / week	1 Hr/Day									
				mean	10.84	8.58	15.83	42.89	49.96	18.46	36.92	846.75
				sd	1.46	.29	.58	1.69	.40	.39	.76	47.39
				n	8	8	8	8	8	8	8	8
		4 / week	4 Hr/Day									
				mean	11.87	8.57	15.63	43.67	50.98	18.29	35.81	827.33
				sd	1.96	.33	.32	1.57	.29	.44	.83	17.47
				n	9	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day									
				mean	13.42	8.81	16.15	44.55	50.57	18.34	36.27	820.27
				sd	2.35	.27	.45	1.64	.75	.36	.79	38.54
n				11	11	11	11	11	11	11	11	

TABLE 12  
L06234 Hematology Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT		
Female	Control	4 / week	4 Hr/Day										
				mean	10.37	8.08	15.71	42.18	52.20	19.47	37.29	769.96	
				sd	1.58	.42	.51	1.99	.62	.68	1.19	48.91	
				n	24	24	24	24	24	24	24	24	
	LowConc	2 / week	1 Hr/Day										
				mean	10.03	8.35	16.03	43.64	52.29	19.22	36.76	833.00	
				sd	1.46	.27	.37	1.35	.40	.57	1.00	58.11	
				n	9	9	9	9	9	9	9	9	
		4 / week	4 Hr/Day										
				mean	10.76	7.99	15.64	41.72	52.18	19.59	37.51	882.00	
				sd	1.49	.24	.42	1.30	.47	.42	.63	47.41	
				n	9	9	9	9	9	9	9	9	
		HighConc	2 / week	4 Hr/Day									
					mean	10.61	8.29	15.62	43.04	51.93	18.86	36.31	832.56
					sd	.88	.30	.45	1.37	.41	.53	.93	56.05
					n	9	9	9	9	9	9	9	9
	4 / week		1 Hr/Day										
				mean	10.53	7.84	15.61	40.59	51.77	19.90	38.48	848.56	
				sd	1.53	.26	.41	1.29	.48	.35	.71	50.97	
				n	9	9	9	9	9	9	9	9	
	PosCont	4 / week	4 Hr/Day										
				mean	12.62	8.20	15.87	42.82	52.23	19.37	37.07	834.33	
				sd	2.24	.29	.45	1.65	.51	.57	1.28	59.34	
				n	12	12	12	12	12	12	12	12	

PART TWO

APPENDIX

SECTION F. HEMATOLOGY: DIFFERENTIAL COUNTS

IIT RESEARCH INSTITUTE

SA/F-1

# 1 L06234 Statistical Report - Hematology Differential

Multivariate analysis of variance models (Bock, 1975) were used to analyze the hematology differential variables: WBC, NRBC, NEUT, LYMPH, MONO, EOS, BASO, and IMNEUT. Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: Sex (male or female), Period (exposure or recovery), concentration (control, 100  $mg/m^3$ , 200  $mg/m^3$ , or positive control at 200  $mg/m^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using a multivariate analysis of variance model which included all five factors and all two way interactions. The second set of analyses concentrated on examining the differences between the dosed and control animals. These analyses were performed separately in each of the four subsamples, defined by period and sex, in order to examine group differences while controlling for the effects of period and sex. First, a one-factor multivariate analysis of variance was performed on the eight hematology differential variables, with concentration as the grouping factor, augmented by simple contrasts which allowed a statistical comparison to be made between each group (100  $mg/m^3$ , 200  $mg/m^3$ , and positive control at 200  $mg/m^3$ ) and the control group. In order to examine the consistency of any group differences between the levels of frequency and duration, a second multivariate analysis of variance was performed. In this second analysis, the low and high dose animals were further grouped depending on their duration and frequency of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration and frequency subgroups.

## 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance yielded a single significant interaction effect of concentration by frequency ( $p < .019$ ). Significant main effects of duration ( $p < .033$ ), sex ( $p < .001$ ) and period ( $p < .001$ ) were also observed. All other main effects and two way interactions were observed to be non-significant by the multivariate test.

The concentration by frequency interaction was observed to be significant only for NRBC levels ( $p < .035$ ) by the univariate F-test. The observed means for the subgroups defined by concentration and frequency, listed in Table 1, illustrate the nature of the interaction for this variable. For low dose animals, NRBC levels were inversely related to the frequency of exposure (i.e., 2 per week had higher levels than 4 per week), whereas the reverse was true for high dose animals. These effects were consistent but small (1/3 sd unit).

The main effect of duration was observed to be statistically significant by the univariate test for the variables: WBC ( $p < .033$ ), NEUT ( $p < .009$ ), MONO ( $p < .016$ ), and EOS ( $p < .013$ ). Table 2 lists the observed means for these variables by duration, and indicates that the 4 hr/day exposure was associated with increased levels on all 4 variables.

The main effect of sex was observed to be statistically significant by the univariate test for the variables: NEUT ( $p < .003$ ), and MONO ( $p < .001$ ). Table 3 lists the observed means for these variables by duration, and indicates that the males were associated with increased levels on both variables.

The main effect of period was observed to be statistically significant by the univariate test for

the variables: WBC ( $p < .001$ ), NRBC ( $p < .003$ ), NEUT ( $p < .001$ ), and LYMPH ( $p < .001$ ). Table 4 lists the observed means for these variables by duration, and indicates that the recovery levels were increased relative to the treatment sacrifice levels on all four variables.

## 1.2 Analysis of All Animals - Comparison to Controls

In the first set of analyses, concentration differences within each of the four subsamples defined by period and sex were examined. The significant differences that were observed are listed in Table 5, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 6.

The univariate tests performed on the male exposure animals revealed significant group differences for WBC ( $p < .016$ ), and NEUT ( $p < .001$ ). As Tables 5 and 6 reveal, the positive control group exhibited significantly elevated means on WBC and NEUT, relative to the control group, and the high dose group exhibited the same result for NEUT.

For the female exposure animals, significant group differences were observed on WBC ( $p < .028$ ), MONO ( $p < .002$ ), and NEUT ( $p < .001$ ). As indicated in Tables 5 and 6, positive controls exhibited significant increases on WBC, MONO and NEUT as compared to controls, whereas high dose animals only exhibited an increase in NEUT.

Turning to the results from the recovery animals, significant group differences for the male recovery animals were observed by the univariate tests on WBC ( $p < .001$ ), NRBC ( $p < .010$ ), NEUT ( $p < .001$ ), and MONO ( $p < .031$ ). Tables 5 and 6 indicate that the positive control group was significantly elevated on each of these variables, relative to the control group. Additionally, the low dose group was significantly elevated on WBC, NEUT and MONO as compared to the controls.

For the female recovery animals, significant group differences were observed on WBC ( $p < .003$ ), NEUT ( $p < .001$ ), MONO ( $p < .001$ ), and EOS ( $p < .001$ ). As indicated in Tables 5 and 6, the positive control group exhibited significant increases on all four variables as compared to the control group.

In the second set of analyses, concentration differences were again examined within each of the four subsamples defined by period and sex. However, in order to also examine the effects of frequency and duration, the low and high dose animals were further divided into subgroups depending on their level of frequency and duration. The significant differences that were observed for the exposure animals are listed in Table 7, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 8. Similarly, the significant differences that were observed for the recovery animals are listed in Table 9, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 10.

The univariate tests performed on the male exposure animals revealed significant group differences in terms of WBC ( $p < .050$ ), and NEUT ( $p < .001$ ). As Tables 7 and 8 reveal, the positive control group exhibited a significantly elevated mean on both variables, relative to the control group, while the high dose group with 2 weekly exposures of 4 hours per day exhibited elevated NEUT, relative to the control group.

For the female exposure animals, significant group differences were observed on MONO ( $p < .005$ ) and NEUT ( $p < .001$ ). As indicated in Tables 7 and 8, positive controls exhibited significant increases on both variables, as compared to controls. Also, the low dose group exposed for 4 hours per day, 4 times per week had significantly increased NEUT, relative to the control group.

Significant group differences for the male recovery animals were observed by the univariate tests on WBC ( $p < .001$ ), NRBC ( $p < .026$ ), NEUT ( $p < .001$ ), and MONO ( $p < .01$ ). Tables 9 and 10 indicate that the positive control group was significantly elevated on all four of these variables

relative to the control group. Additionally, low dose animals exposed for 4 hrs 2 times per week showed increases in WBC, NEUT, and MONO. High dose animals exposed for 1 hr twice a week exhibited elevated MONO, and high dose animals exposed for 4 hrs 4 times per week exhibited elevated WBC relative to controls.

For the female recovery animals, significant group differences were observed on WBC ( $p < .013$ ), NEUT ( $p < .001$ ), MONO ( $p < .001$ ), and EOS ( $p < .001$ ). As indicated in Tables 9 and 10, the positive control group was significantly elevated on all four of these variables relative to the control group. Additionally, low dose animals exposed for 4 hrs 4 times per week showed increases in NEUT. Low dose animals exposed for 1 hr twice a week exhibited decreased MONO, relative to controls.

### 1.3 Summary

In general, duration and frequency did not play a major role in the effect of exposure to the compound. Both male and female high dose animals exhibited significantly increased NEUT levels relative to controls, but this effect was not present following the recovery period.

## References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.



TABLE 1  
 L06234 Hematology Differential Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Frequency Subgroups  
 (Averaging over Duration, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	FREQUENCY	NRBC
LowConc	2 / week	
	4 / week	
HighConc	2 / week	
	4 / week	
OVERALL	mean	

TABLE 2  
 L06234 Hematology Differential Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Duration Subgroups  
 (Averaging over Concentration, Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

DURATION		WBC	NEUT	MONO	EOS
1 Hr/Day					
	mean	10.04	1.44	.27	.06
	sd	1.73	.42	.13	.06
	n	69	69	69	69
4 Hr/Day					
	mean	10.74	1.68	.32	.09
	sd	2.05	.62	.12	.08
	n	72	72	72	72
OVERALL	mean	10.40	1.56	.30	.08
	sd	1.93	.54	.13	.07
	n	141	141	141	141

TABLE 3  
 L06234 Hematology Differential Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Period)  
 (variables with significant univariate F-test results)

SEX	NEUT	MONO
Male		
mean	1.69	.34
sd	.58	.14
n	69	69
Female		
mean	1.44	.26
sd	.49	.11
n	72	72
OVERALL mean	1.56	.30
sd	.54	.13
n	141	141

TABLE 4  
 L06234 Hematology Differential Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	WBC	NRBC	NEUT	LYMPH
Post-Exp				
mean	9.77	.03	1.40	8.00
sd	1.92	.06	.39	1.79
n	70	70	70	70
Post-Rec				
mean	11.02	.07	1.73	8.92
sd	1.73	.09	.62	1.40
n	71	71	71	71
OVERALL mean	10.40	.05	1.56	8.46
sd	1.93	.08	.54	1.67
n	141	141	141	141

TABLE 5  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Period and Sex*  
(averaging over frequency and duration)

	concentration	significant increases	significant decreases
<i>Male Post-Exposure</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>	3	
	positive control	1,3	
<i>Male Post-Recovery</i>	100 mg/m <sup>3</sup>	1,3,5	
	200 mg/m <sup>3</sup>		
	positive control	1,2,3,5	
<i>Female Post-Exposure</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>	3	
	positive control	1,3,5	
<i>Female Post-Recovery</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>		
	positive control	1,3,5,6	

*Key for significant effects*

1 = WBC, 2 = NRBC, 3 = NEUT, 4 = LYMPH,  
5 = MONO, 6 = EOS, 7 = BASO, 8 = IMNEUT

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 6  
L06234 Hematology Differential Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT
Male	Post-Exp	Control								
		mean	9.10	.05	1.25	7.50	.29	.06	0.00	0.00
		sd	2.15	.08	.51	1.96	.17	.09	0.00	0.00
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	9.62	.02	1.44	7.79	.31	.09	0.00	0.00
		sd	1.60	.04	.33	1.40	.13	.07	0.00	0.00
		n	16	16	16	16	16	16	16	16
		HighConc								
		mean	9.59	.03	1.55	7.66	.33	.07	0.00	0.00
		sd	2.10	.06	.42	2.00	.13	.07	0.00	0.00
		n	18	18	18	18	18	18	18	18
	PosCont									
	mean	11.33	.03	2.24	8.58	.40	.11	0.00	0.00	
	sd	1.53	.05	.61	1.36	.19	.09	0.00	0.00	
	n	12	12	12	12	12	12	12	12	
	Post-Rec	Control								
		mean	10.43	.07	1.54	8.55	.28	.05	0.00	0.00
		sd	1.49	.08	.36	1.33	.14	.05	0.00	0.00
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	11.75	.04	1.96	9.33	.38	.08	0.00	0.00
		sd	2.09	.05	.79	1.73	.12	.09	0.00	0.00
		n	18	18	18	18	18	18	18	18
HighConc										
mean		11.38	.07	1.79	9.17	.35	.07	0.00	0.00	
sd		1.77	.09	.54	1.42	.17	.09	0.00	0.00	
n		17	17	17	17	17	17	17	17	
PosCont										
mean	13.42	.20	2.81	10.12	.43	.07	0.00	0.00		
sd	2.35	.24	.46	1.90	.21	.06	0.00	0.00		
n	11	11	11	11	11	11	11	11		

TABLE 6  
L06234 Hematology Differential Analysis of All Animals  
Means, SDs, and Ns for Sex by Period Subgroups  
(Averaging over Frequency and Duration)

SEX	PERIOD	GROUP	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT
Female	Post-Exp	Control								
		mean	9.75	.05	1.07	8.38	.26	.05	0.00	0.00
		sd	2.01	.08	.42	1.75	.12	.06	0.00	0.00
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	10.07	.02	1.26	8.45	.27	.09	0.00	0.00
		sd	1.81	.04	.42	1.64	.09	.07	0.00	0.00
		n	18	18	18	18	18	18	18	18
		HighConc								
		mean	9.80	.05	1.34	8.08	.29	.07	0.00	0.00
		sd	2.23	.09	.36	2.05	.13	.08	0.00	0.00
		n	18	18	18	18	18	18	18	18
	PosCont									
	mean	11.94	.12	2.48	8.89	.44	.11	0.00	0.00	
	sd	1.96	.16	.58	1.66	.17	.07	0.00	0.00	
	n	12	12	12	12	12	12	12	12	
	Post-Rec	Control								
		mean	10.37	.10	1.37	8.67	.29	.04	0.00	0.00
		sd	1.58	.13	.44	1.48	.15	.05	0.00	0.00
		n	24	24	24	24	24	24	24	24
		LowConc								
		mean	10.39	.08	1.64	8.44	.23	.07	0.00	0.00
		sd	1.48	.08	.64	1.18	.10	.06	0.00	0.00
		n	18	18	18	18	18	18	18	18
HighConc										
mean		10.57	.08	1.53	8.73	.23	.06	0.00	0.00	
sd		1.21	.11	.42	1.15	.09	.05	0.00	0.00	
n		18	18	18	18	18	18	18	18	
PosCont										
mean	12.62	.15	3.00	9.02	.44	.15	0.00	0.00		
sd	2.24	.12	.86	1.66	.18	.11	0.00	0.00		
n	12	12	12	12	12	12	12	12		

TABLE 7  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Exposure Animals by Frequency and Duration*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	M		
100 mg/m <sup>3</sup>	2	4	F		
100 mg/m <sup>3</sup>	4	1	F		
100 mg/m <sup>3</sup>	4	4	M		
200 mg/m <sup>3</sup>	2	1	F		
200 mg/m <sup>3</sup>	2	4	M	3	
200 mg/m <sup>3</sup>	4	1	M		
200 mg/m <sup>3</sup>	4	4	F	<b>3</b>	
positive control			M	1,3	
positive control			F	<b>3,5</b>	

*Key for significant effects*

1 = WBC, 2 = NRBC, 3 = NEUT, 4 = LYMPH,  
5 = MONO, 6 = EOS, 7 = BASO, 8 = IMNEUT

normal text:  $p < .05$ , bold text:  $p < .01$



TABLE 8  
L06234 Hematology Differential Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT		
Male	Control	4 / week	4 Hr/Day	mean	9.10	.05	1.25	7.50	.29	.06	0.00	0.00	
				sd	2.15	.08	.51	1.96	.17	.09	0.00	0.00	
				n	24	24	24	24	24	24	24	24	
		LowConc	2 / week	1 Hr/Day	mean	9.54	.01	1.41	7.79	.29	.09	0.00	0.00
					sd	1.50	.04	.29	1.17	.12	.07	0.00	0.00
					n	7	7	7	7	7	7	7	7
			4 / week	4 Hr/Day	mean	9.68	.02	1.47	7.79	.33	.09	0.00	0.00
	sd				1.75	.04	.37	1.62	.14	.08	0.00	0.00	
	n				9	9	9	9	9	9	9	9	
	HighConc	2 / week	4 Hr/Day	mean	10.10	.02	1.59	8.10	.33	.11	0.00	0.00	
				sd	2.56	.07	.52	2.46	.13	.07	0.00	0.00	
				n	9	9	9	9	9	9	9	9	
		4 / week	1 Hr/Day	mean	9.09	.03	1.51	7.21	.32	.04	0.00	0.00	
				sd	1.49	.05	.30	1.41	.14	.05	0.00	0.00	
				n	9	9	9	9	9	9	9	9	
	PosCont	4 / week	4 Hr/Day	mean	11.33	.03	2.24	8.58	.40	.11	0.00	0.00	
				sd	1.53	.05	.61	1.36	.19	.09	0.00	0.00	
n				12	12	12	12	12	12	12	12		

TABLE 8  
L06234 Hematology Differential Analysis of Post-Exposure Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT
Female	Control	4 / week	4 Hr/Day	mean	9.75	.05	1.07	8.38	.26	.05	0.00
				sd	2.01	.08	.42	1.75	.12	.06	0.00
				n	24	24	24	24	24	24	24
	LowConc	2 / week	4 Hr/Day	mean	9.81	.03	1.38	8.06	.28	.09	0.00
				sd	1.98	.05	.49	1.64	.10	.06	0.00
				n	9	9	9	9	9	9	9
		4 / week	1 Hr/Day	mean	10.32	.01	1.13	8.84	.26	.09	0.00
				sd	1.70	.03	.32	1.63	.09	.08	0.00
				n	9	9	9	9	9	9	9
	HighConc	2 / week	1 Hr/Day	mean	9.26	.06	1.20	7.73	.26	.07	0.00
				sd	2.13	.10	.33	2.20	.09	.07	0.00
				n	9	9	9	9	9	9	9
		4 / week	4 Hr/Day	mean	10.34	.04	1.49	8.43	.33	.08	0.00
				sd	2.31	.07	.34	1.94	.16	.08	0.00
				n	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day	mean	11.94	.12	2.48	8.89	.44	.11	0.00
				sd	1.96	.16	.58	1.66	.17	.07	0.00
				n	12	12	12	12	12	12	12

TABLE 9  
Significant Differences for Exposed Animals as Compared to Sex-Matched Filtered-Air Controls  
*Post-Recovery Animals by Duration and Frequency*

concentration	frequency (exp/week)	duration (hr/day)	sex	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	F		5
100 mg/m <sup>3</sup>	2	4	M	<b>1,3,5</b>	
100 mg/m <sup>3</sup>	4	1	M		
100 mg/m <sup>3</sup>	4	4	F	3	
200 mg/m <sup>3</sup>	2	1	M	5	
200 mg/m <sup>3</sup>	2	4	F		
200 mg/m <sup>3</sup>	4	1	F		
200 mg/m <sup>3</sup>	4	4	M	1	
positive control			M	<b>1,2,3,5</b>	
positive control			F	<b>1,3,5,6</b>	

*Key for significant effects*

1 = WBC, 2 = NRBC, 3 = NEUT, 4 = LYMPH,  
5 = MONO, 6 = EOS, 7 = BASO, 8 = IMNEUT

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 10  
L06234 Hematology Differential Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT	
Male	Control	4 / week	4 Hr/Day									
				mean	10.43	.07	1.54	8.55	.28	.05	0.00	0.00
				sd	1.49	.08	.36	1.33	.14	.05	0.00	0.00
				n	24	24	24	24	24	24	24	24
		LowConc	2 / week	4 Hr/Day								
					mean	12.78	.04	2.34	9.87	.44	.11	0.00
	sd				1.60	.05	.88	1.43	.10	.09	0.00	0.00
	n				9	9	9	9	9	9	9	9
	4 / week		1 Hr/Day									
				mean	10.72	.04	1.57	8.80	.31	.06	0.00	0.00
		sd		2.08	.05	.47	1.91	.09	.07	0.00	0.00	
		n		9	9	9	9	9	9	9	9	
		HighConc	2 / week	1 Hr/Day								
					mean	10.84	.04	1.71	8.66	.41	.04	0.00
	sd				1.46	.07	.52	.89	.20	.05	0.00	0.00
	n				8	8	8	8	8	8	8	8
	4 / week		4 Hr/Day									
				mean	11.87	.10	1.86	9.62	.29	.10	0.00	0.00
		sd		1.96	.10	.57	1.69	.12	.11	0.00	0.00	
		n		9	9	9	9	9	9	9	9	
		PosCont	4 / week	4 Hr/Day								
					mean	13.42	.20	2.81	10.12	.43	.07	0.00
	sd				2.35	.24	.46	1.90	.21	.06	0.00	0.00
	n				11	11	11	11	11	11	11	11

TABLE 10  
L06234 Hematology Differential Analysis of Post-Recovery Animals  
Means, SDs, and Ns for All Subgroups

SEX	GROUP	FREQUENCY	DURATION	WBC	NRBC	NEUT	LYMPH	MONO	EOS	BASO	IMNEUT	
Female	Control	4 / week	4 Hr/Day	mean	10.37	.10	1.37	8.67	.29	.04	0.00	0.00
				sd	1.58	.13	.44	1.48	.15	.05	0.00	0.00
				n	24	24	24	24	24	24	24	24
	LowConc	2 / week	1 Hr/Day	mean	10.03	.10	1.40	8.39	.17	.06	0.00	0.00
				sd	1.46	.09	.45	1.16	.07	.05	0.00	0.00
				n	9	9	9	9	9	9	9	9
		4 / week	4 Hr/Day	mean	10.76	.06	1.88	8.50	.30	.09	0.00	0.00
				sd	1.49	.07	.73	1.27	.09	.06	0.00	0.00
				n	9	9	9	9	9	9	9	9
	HighConc	2 / week	4 Hr/Day	mean	10.61	.03	1.42	8.84	.27	.07	0.00	0.00
				sd	.88	.05	.41	1.00	.10	.05	0.00	0.00
				n	9	9	9	9	9	9	9	9
		4 / week	1 Hr/Day	mean	10.53	.12	1.63	8.62	.20	.04	0.00	0.00
				sd	1.53	.14	.42	1.34	.07	.05	0.00	0.00
				n	9	9	9	9	9	9	9	9
	PosCont	4 / week	4 Hr/Day	mean	12.62	.15	3.00	9.02	.44	.15	0.00	0.00
				sd	2.24	.12	.86	1.66	.18	.11	0.00	0.00
				n	12	12	12	12	12	12	12	12

PART TWO  
APPENDIX

SECTION G. PULMONARY LAVAGE

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# 1 L06234 Statistical Report - Pulmonary Lavage

Multivariate analysis of variance models (Bock, 1975) were used to analyze the pulmonary lavage variables: TOTVCELL, TOTCELL, PERVCELL, MONO, LYMPH, NEUT, PHAGO, STDPHAGO, and PROTEIN. Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: Sex (male or female), Period (exposure or recovery), concentration (control, 100 mg/m<sup>3</sup>, 200 mg/m<sup>3</sup>, or positive control at 200 mg/m<sup>3</sup>), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using a multivariate analysis of variance model which included all five factors and all two way interactions.

The second set of analyses concentrated on examining the differences between the dosed and control animals. First, a one-factor multivariate analysis of variance was performed on the nine pulmonary lavage variables for the exposure and recovery animals separately, with concentration as the grouping factor, augmented by simple contrasts. This allowed a statistical comparison to be made between each group (100 mg/m<sup>3</sup>, 200 mg/m<sup>3</sup>, and positive control at 200 mg/m<sup>3</sup>) and the control group for the exposure and recovery animals separately. In order to examine the consistency of any group differences between the levels of duration, a second multivariate analysis of variance was performed, again, separately for the exposure and recovery animals. In this second analysis, the low and high dose animals were further grouped depending on their duration of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration subgroups. A third multivariate analysis of variance was performed for the exposure and recovery animals in order to examine the consistency of any group differences between the levels of frequency. In this analysis, the low and high dose animals were further grouped depending on their frequency of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the frequency subgroups. A final multivariate analysis of variance was performed, combining the exposure and recovery animals, to examine the consistency of any group differences between the levels of both frequency and duration. In this analysis, the low and high dose animals were further grouped depending on both their duration and frequency of exposure. The one-way multivariate analysis of variance was augmented with simple contrasts to examine the possibility of a group by duration by frequency interaction.

## 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance yielded significant interaction effects of duration by frequency, concentration by frequency, sex by frequency, concentration by duration, sex by duration, sex by concentration, and period by duration (all  $p < .001$  except period by duration  $p < .032$ ) by the multivariate test. Significant main effects of frequency ( $p < .039$ ), duration ( $p < .001$ ), concentration ( $p < .011$ ), and period ( $p < .001$ ) were also observed. All other main effects and two way interactions were observed to be non-significant by the multivariate test.

The duration by frequency interaction was observed to be significant for PHAGO ( $p < .036$ ) and PROTEIN ( $p < .010$ ) by the univariate F-tests. The observed means for the subgroups defined by duration and frequency, listed in Table 1, illustrate the nature of the duration by frequency

interaction for these variables. For the frequency of 2 per week, 4 hour per day exposures elevated PHAGO values moderately and had little effect on PROTEIN values, relative to 1 hour per day exposures. In contrast, for the frequency of 4 per week, 4 hour per day exposures decreased PHAGO values and greatly increased PROTEIN values, relative to 1 hour per day exposures.

The sex by frequency interaction was observed to be significant only for STDPHAGO ( $p < .007$ ), while the concentration by frequency interaction was not observed to be significant for any of the nine lavage parameters, by the univariate F-tests. The observed means for the subgroups defined by sex and frequency, listed in Table 2, illustrate the nature of the sex by frequency interaction for STDPHAGO. Males exhibited a decrease in STDPHAGO values with 4 exposures per week, relative to 2 per week, whereas, females increased slightly when exposed 4 times per week, relative to 2 exposures per week.

Regarding the concentration by duration interaction, significant effects were observed for LYMPH, STDPHAGO, PROTEIN ( $p < .001$  for all), and MONO ( $p < .014$ ) by the subsequent univariate F-tests. The observed means for the subgroups defined by concentration and duration are given in Table 3. These observed means indicate that for the low dose animals, exposures of 4 hours per day elevated LYMPH and STDPHAGO values, relative to exposures of 1 hour per day, whereas for the high dose animals the reverse was observed: exposures of 4 hours per day decreased LYMPH and STDPHAGO values, relative to 1 hour per day exposures. Also, exposures of 4 hours per day elevated PROTEIN values and decreased MONO values, relative to 1 hour per day exposures, however, this effect was more pronounced among the low dose animals than the high dose animals.

For the sex by duration interaction, significant effects were observed for PHAGO ( $p < .001$ ) and PROTEIN ( $p < .030$ ) by the subsequent univariate F-tests. The observed means for the subgroups defined by sex and duration are given in Table 4. These observed means indicate that for male animals, exposures of 4 hours per day elevated PHAGO values, relative to exposures of 1 hour per day, whereas for the female animals the reverse was observed: exposures of 4 hours per day decreased PHAGO values, relative to 1 hour per day exposures. Exposures of 4 hours per day elevated PROTEIN values, relative to 1 hour per day exposures, however, this effect was more pronounced among the female animals than the male animals.

The sex by concentration interaction was observed to be significant only for PHAGO ( $p < .001$ ) by the univariate F-tests. The observed means for the subgroups defined by sex and concentration are listed in Table 5. High dose males exhibited an increase in PHAGO values, relative to low dose males, whereas, high dose females were decreased, relative to low dose females.

The period by duration interaction was observed to be significant for TOTVCELL ( $p < .028$ ) and TOTCELL ( $p < .037$ ) by the univariate F-tests. The observed means for the subgroups defined by period and duration, listed in Table 6, illustrate the nature of the period by duration interaction for these variables. Although, overall, the duration of 4 hours per day increased TOTVCELL and TOTCELL values, relative to the duration of 1 hour per day, this increase was more pronounced during the exposure period than the recovery period.

The main effect of frequency was observed to be statistically significant by the univariate test for the variables: MONO, NEUT ( $p < .001$  for both), PHAGO ( $p < .037$ ), and STDPHAGO ( $p < .046$ ). Table 7 which lists the observed means for these variables by frequency indicates that animals with 4 exposures per week had an elevated NEUT mean, and decreased MONO, PHAGO, and STDPHAGO means, relative to animals exposed twice per week.

The main effect of duration was observed to be statistically significant by the univariate test for the variables: TOTVCELL, TOTCELL, MONO, NEUT, and PROTEIN ( $p < .001$  for all). Table 8 which lists the observed means for these variables by duration indicates that animals with exposures of 4 hours per day had elevated TOTVCELL, TOTCELL, NEUT and PROTEIN means, and a decreased MONO means, relative to animals with 1 hour per day exposures.



The main effect of concentration was observed to be statistically significant by the univariate test for the variables: MONO, NEUT, and PROTEIN ( $p < .001$  for MONO and NEUT,  $p < .002$  for PROTEIN). Table 9 which lists the observed means for these variables by concentration indicates that the high dose group had elevated NEUT and PROTEIN means, and a decreased MONO mean, relative to the low dose group.

Finally, the main effect of period was observed to be statistically significant by the univariate test for the following pulmonary lavage parameters: TOTVCELL ( $p < .007$ ), TOTCELL ( $p < .012$ ), PERVCELL ( $p < .018$ ), LYMPH ( $p < .001$ ), NEUT ( $p < .001$ ), PHAGO ( $p < .001$ ), and PROTEIN ( $p < .001$ ). Table 10 which lists the observed means for these variables by period indicates that the exposure animals had an elevated mean only on PHAGO, and decreased means on TOTVCELL, TOTCELL, PERVCELL, LYMPH, and PROTEIN, relative to the recovery animals.

## 1.2 Analysis of All Animals - Comparison to Controls

In the first set of analyses, concentration differences were examined separately for the exposure and recovery animals. The significant differences that were observed are listed in Table 11, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 12.

The univariate tests performed on the exposure animals revealed significant group differences in terms of TOTVCELL, TOTCELL, PERVCELL, MONO, NEUT, and PROTEIN ( $p < .001$  for all except  $p < .046$  for PERVCELL). As Tables 11 and 12 reveal, the positive control group exhibited significantly elevated means on TOTVCELL, TOTCELL, NEUT, and PROTEIN, and a significantly decreased mean on MONO, relative to the control group. Also, the high dose group exhibited significantly elevated means on NEUT and MONO, and a significantly decreased mean on MONO, relative to the control group. Finally, the low dose group was significantly elevated in terms of NEUT, relative to the controls.

Turning to the results from the recovery animals, significant group differences were observed by the univariate tests on TOTVCELL, TOTCELL, PERVCELL, MONO, NEUT and PROTEIN ( $p < .001$  for all). Tables 11 and 12 indicate that the positive control group was significantly elevated on TOTVCELL, TOTCELL, NEUT, and PROTEIN, and significantly decreased on PERVCELL and MONO, relative to the control group. Additionally, both low and high dose groups were significantly elevated on TOTVCELL and TOTCELL, while the high dose group was also significantly elevated on NEUT, and significantly decreased on MONO, as compared to the controls.

In the second set of analyses, concentration differences were again examined separately for exposure and recovery animals. However, in order to also examine the effect of duration, the low and high dose animals were further divided into subgroups depending on their duration level. The significant differences that were observed for the exposure and recovery animals are listed in Table 13, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 14.

The univariate tests performed on the exposure animals revealed significant group differences in terms of TOTVCELL, TOTCELL, PERVCELL, MONO, LYMPH, NEUT, and PROTEIN ( $p < .001$  for all, except,  $p < .043$  for PERVCELL and  $p < .009$  for LYMPH). As Tables 13 and 14 reveal, all groups, with the exception of the low dose group with exposure duration of 1 hour per day, exhibited increased NEUT and decreased MONO means, relative to the control group. Additionally, the positive control group and both dosed groups with 4 hour per day exposures had elevated PROTEIN means, relative to the controls. TOTVCELL and TOTCELL means were significantly elevated for the positive control group and for the high dose group with increased exposures (4 hr/day), relative to the controls. Finally, the low dose group with increased exposure

(4 hr/day) exhibited an elevated PERVCELL mean, while the low dose group with decreased exposure (1 hr/day) exhibited a decreased LYMPH mean, relative to the control group.

Significant group differences on the recovery animals were observed by the univariate tests on TOTVCELL, TOTCELL, PERVCELL, MONO, NEUT, STDPHAGO, and PROTEIN ( $p < .001$  for all, except  $p < .003$  for STDPHAGO). Tables 13 and 14 indicate that the positive control group and both of the dosed groups with 4 daily exposures were significantly elevated on TOTVCELL, TOTCELL, and NEUT, relative to the control group. Additionally, both the positive control group and the high dose group with 4 daily exposures exhibited decreased means on MONO and STDPHAGO, relative to the controls. Also, the positive control group exhibited an increased PROTEIN and a decreased PERVCELL mean, relative to the control group.

In the next set of analyses, concentration differences were again examined separately for exposure and recovery animals, however, in order to also examine the effect of frequency, the low and high dose animals were further divided into subgroups depending on their frequency level. The significant differences that were observed for the exposure and recovery animals are listed in Table 15, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 16.

The univariate tests performed on the exposure animals revealed significant group differences in terms of TOTVCELL, TOTCELL, MONO, NEUT, and PROTEIN ( $p < .001$  for all). As Tables 15 and 16 reveal, all groups, with the exception of the low dose group with exposure frequency of 2 per week, exhibited increased NEUT and decreased MONO means, relative to the control group. Additionally, the positive control group and the high dose group with 4 weekly exposures had elevated PROTEIN means, relative to the controls. TOTVCELL and TOTCELL means were significantly elevated for the positive control group, relative to the controls.

Significant group differences on the recovery animals were observed by the univariate tests on TOTVCELL, TOTCELL, PERVCELL, MONO, NEUT, and PROTEIN ( $p < .001$  for all, except  $p < .002$  for TOTVCELL). Tables 15 and 16 indicate that the positive control group and both of the dosed groups with 4 weekly exposures were significantly elevated on TOTVCELL and TOTCELL, relative to the control group. Also, the positive control group exhibited increased NEUT and PROTEIN means, and decreased PERVCELL and MONO means, relative to the control group.

In the final set of analyses, concentration differences were examined combining the exposure and recovery animals, in order to examine the interaction effect of duration and frequency. Here, the low and high dose animals were further divided into subgroups depending on both their frequency and duration level. The significant differences that were observed are listed in Table 17, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 18.

The univariate tests performed revealed significant group differences in terms of TOTVCELL, TOTCELL, PERVCELL, MONO, NEUT, STDPHAGO, and PROTEIN ( $p < .001$  for all, except  $p < .003$  for PERVCELL and STDPHAGO). As Tables 17 and 18 reveal, all groups, with the exception of the low dose group with exposure duration of 1 hour per day, exhibited increased NEUT and decreased MONO means, relative to the control group. Additionally, the positive control group and three dosed groups (both low and high dose groups with 4 weekly exposures 4 times per day, as well as the high dose group with 2 weekly exposures once a day) exhibited elevated PROTEIN means, relative to the controls. TOTVCELL and TOTCELL means were significantly elevated for the positive control group and for both high dose groups with 4 exposures per day, relative to the controls. The low dose group with 2 weekly exposures once daily had an elevated MONO mean and a decreased NEUT mean, while the low dose group exposed biweekly 4 hours per day exhibited elevated TOTVCELL and PERVCELL means, relative to the control group. Finally, both the high dose group with 4 weekly exposures of 4 hour per day and the positive control group exhibited decreased STDPHAGO means, relative to the control group, with the positive control group also

exhibiting a decreased PERVCELL mean. Significant group differences on the recovery animals were observed by the univariate tests on TOTVCELL, TOTCELL, PERVCELL, MONO, NEUT, and PROTEIN ( $p < .001$  for all, except  $p < .002$  for TOTVCELL). Tables 15 and 16 indicate that the positive control group and both of the dosed groups with 4 weekly exposures were significantly elevated on TOTVCELL and TOTCELL, relative to the control group. Also, the positive control group exhibited increased NEUT and PROTEIN means, and decreased PERVCELL and MONO means, relative to the control group.

### 1.3 Summary

Overall, low dose exposure animals exhibited significantly increased NEUT relative to controls (see Table 11). At the high dose, both NEUT and PROTEIN were elevated and significant decreases were observed for MONO. These effects appeared to be most pronounced for the 4 hour per day duration (Table 13) and 4 time per week exposure frequency (Table 15). In fact, in high dose animals virtually identical significant differences were observed for 4 hour per week 4 time per week animals as for positive controls (Table 17). For the low duration and low frequency conditions, both high and low dose animals appeared to normalize following recovery, but this was not true for positive controls or high duration and/or high frequency animals.

### References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971

TABLE 1  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Frequency by Duration Subgroups  
 (Averaging over Concentration, Period, and Sex)  
 (variables with significant univariate F-test results)

FREQUENCY	DURATION	PHAGO	PROTEIN
2 / week	1 Hr/Day		
		mean	28557.20
		sd	121.44
		n	107.76
	4 Hr/Day		
		mean	28557.20
		sd	121.44
		n	107.76
4 / week	1 Hr/Day		
		mean	29137.74
		sd	91.46
		n	53.48
	4 Hr/Day		
		mean	26198.92
		sd	199.70
		n	52.67
OVERALL	mean	28508.03	134.34
	sd	4839.65	80.87
	n	48	48

TABLE 2  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	FREQUENCY	STDPHAGO	
Male	2 / week		
		mean	51538.83
		sd	5070.23
		n	12
	4 / week		
		mean	47537.34
		sd	2669.63
		n	12
	Female	2 / week	
			mean
sd			2594.50
n			12
4 / week			
		mean	49175.97
		sd	3304.58
		n	12
OVERALL		mean	49196.35
		sd	3745.92
	n	48	

TABLE 3  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Duration Subgroups  
 (Averaging over Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	DURATION	MONO	LYMPH	STDPHAGO	PROTEIN		
LowConc	1 Hr/Day						
		mean	95.33	1.17	47936.97	61.64	
		sd	8.02	3.21	2974.02	46.98	
		n	12	12	12	12	
	4 Hr/Day						
		mean	79.25	3.92	51374.51	166.19	
		sd	10.96	2.87	4751.71	60.12	
		n	12	12	12	12	
	HighConc	1 Hr/Day					
			mean	84.75	4.25	51134.63	151.26
			sd	7.17	4.35	2035.47	91.51
			n	12	12	12	12
4 Hr/Day							
		mean	73.08	2.08	46339.30	158.27	
		sd	11.33	3.06	2116.29	77.06	
		n	12	12	12	12	
OVERALL		mean	83.10	2.85	49196.35	134.34	
		sd	12.39	3.55	3745.92	80.87	
		n	48	48	48	48	

TABLE 4  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Duration Subgroups  
 (Averaging over Concentration, Frequency, and Period)  
 (variables with significant univariate F-test results)

SEX	DURATION	PHAGO	PROTEIN
Male	1 Hr/Day		
		mean	27494.39
		sd	136.24
		n	99.63
	4 Hr/Day		
		mean	30205.13
		sd	163.35
		n	76.96
Female	1 Hr/Day		
		mean	30200.55
		sd	76.66
		n	55.68
	4 Hr/Day		
		mean	26132.03
		sd	161.11
		n	60.51
OVERALL		mean	28508.03
		sd	134.34
		n	80.87

TABLE 5  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Concentration Subgroups  
 (Averaging over Duration, Frequency and Period)  
 (variables with significant univariate F-test results)

SEX	CONCENTRATION	PHAGO
Male	LowConc	
		mean 26942.14
		sd 4028.88
		n 12
	HighConc	
		mean 30757.38
		sd 4381.16
		n 12
Female	LowConc	
		mean 30992.69
		sd 5572.77
		n 12
	HighConc	
		mean 25339.90
		sd 2777.74
		n 12
OVERALL	mean	28508.03
	sd	4839.65
	n	48



TABLE 6  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period by Duration Subgroups  
 (Averaging over Concentration, Frequency, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	DURATION	TOTVCELL	TOTCELL
Post-Exp	1 Hr/Day		
		mean	12695833.3
		sd	2059949.1
		n	12
	4 Hr/Day		
		mean	19325000.0
		sd	3141764.1
		n	12
Post-Rec	1 Hr/Day		
		mean	17393750.0
		sd	4463540.7
		n	12
	4 Hr/Day		
		mean	20256250.0
		sd	4702539.5
		n	12
OVERALL	mean	17417708.3	17795312.5
	sd	4671242.3	4734287.2
	n	48	48

TABLE 7  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Frequency Subgroups  
 (Averaging over Concentration, Duration, Period, and Sex)  
 (variables with significant univariate F-test results)

FREQUENCY	MONO	NEUT	PHAGO	STDPHAGO
2 / week				
mean	87.71	9.63	29347.72	50036.05
sd	10.23	9.05	4474.02	4227.37
n	24	24	24	24
4 / week				
mean	78.50	18.46	27668.33	48356.66
sd	12.84	12.87	5135.60	3054.79
n	24	24	24	24
OVERALL mean	83.10	14.04	28508.03	49196.35
sd	12.39	11.88	4839.65	3745.92
n	48	48	48	48

TABLE 8  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Duration Subgroups  
 (Averaging over Concentration, Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

DURATION	TOTVCELL	TOTCELL	MONO	NEUT	PROTEIN
1 Hr/Day					
mean	15044791.7	15422916.7	90.04	7.25	106.45
sd	4161181.8	4178145.2	9.20	7.59	84.59
n	24	24	24	24	24
4 Hr/Day					
mean	19790625.0	20167708.3	76.17	20.83	162.23
sd	3939948.8	4074139.5	11.35	11.59	67.71
n	24	24	24	24	24
OVERALL mean	17417708.3	17795312.5	83.10	14.04	134.34
sd	4671242.3	4734287.2	12.39	11.88	80.87
n	48	48	48	48	48

TABLE 9  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration Subgroups  
 (Averaging over Duration, Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

Concentration	MONO	NEUT	PROTEIN
LowConc			
mean	87.29	10.17	113.92
sd	12.48	10.45	75.07
n	24	24	24
HighConc			
mean	78.92	17.92	154.76
sd	11.03	12.15	82.81
n	24	24	24
OVERALL mean	83.10	14.04	134.34
sd	12.39	11.88	80.87
n	48	48	48

TABLE 10  
 L06234 Pulmonary Lavage Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Duration, Frequency, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	TOTVCELL	TOTCELL	PERVCELL	LYMPH	PHAGO	PROTEIN
Post-Exp						
mean	16010416.7	16471875.0	97.15	1.00	30745.93	100.89
sd	4267826.5	4333021.0	2.22	1.44	4784.74	65.97
n	24	24	24	24	24	24
Post-Rec						
mean	18825000.0	19118750.0	98.52	4.71	26270.12	167.79
sd	4716166.7	4834366.6	1.47	4.07	3810.12	81.71
n	24	24	24	24	24	24
OVERALL mean	17417708.3	17795312.5	97.83	2.85	28508.03	134.34
sd	4671242.3	4734287.2	1.99	3.55	4839.65	80.87
n	48	48	48	48	48	48

TABLE 11  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Period*  
(averaging over sex, frequency and duration)

	concentration	significant increases	significant decreases
<i>Post-Exposure</i>	100 <i>mg/m</i> <sup>3</sup>	<b>6</b>	
	200 <i>mg/m</i> <sup>3</sup>	<b>6,9</b>	<b>4</b>
	positive control	<b>1,2,6,9</b>	<b>4</b>
<i>Post-Recovery</i>	100 <i>mg/m</i> <sup>3</sup>	<b>1,2</b>	
	200 <i>mg/m</i> <sup>3</sup>	<b>1,2,6</b>	<b>4</b>
	positive control	<b>1,2,6,9</b>	<b>3,4</b>

*Key for significant effects*

1 = TOTVCELL, 2 = TOTCELL, 3 = PERVCELL,  
4 = MONO, 5 = LYMPH, 6 = NEUT, 7 = PHAGO,  
8 = STDPHAGO, 9 = PROTEIN

normal text: *p* < .05, **bold text**: *p* < .01

TABLE 12  
L06234 Pulmonary Lavage Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration Subgroups  
(averaging over sex, frequency and duration)

PERIOD	GROUP	TOTVCELL	TOTCELL	PERVCELL	MONO	LYMPH	NEUT	PHAGO	STDPHAGO	PROTEIN
Post-Exp	Control									
	mean	14920313	15675000	95.00	97.06	1.31	1.63	31959.39	50000.00	63.44
	sd	6708957	6921055	2.91	3.73	1.66	3.18	4399.50	2041.90	55.61
	n	16	16	16	16	16	16	16	16	16
	LowConc									
	mean	14550000	14979167	97.05	88.92	1.00	10.08	31034.44	49075.05	82.58
	sd	2803873	2789995	2.57	12.74	1.65	11.45	4812.74	1957.74	62.97
	n	12	12	12	12	12	12	12	12	12
	HighConc									
	mean	17470833	17964583	97.24	77.17	1.00	21.83	30457.43	48498.04	119.20
	sd	5056621	5158625	1.93	13.48	1.28	14.05	4952.25	2756.46	66.36
	n	12	12	12	12	12	12	12	12	12
	PosCont									
	mean	82171875	87187500	94.42	36.88	3.00	60.13	29945.19	47985.80	504.70
	sd	19864321	21329840	2.86	7.49	2.83	9.06	6908.64	6701.35	138.97
	n	8	8	8	8	8	8	8	8	8
Post-Rec	Control									
	mean	14578125	14728125	99.05	88.56	3.81	7.63	26663.96	50000.00	112.49
	sd	2943961	3025501	1.34	9.97	3.08	7.46	6250.00	4766.88	44.77
	n	16	16	16	16	16	16	16	16	16
	LowConc									
	mean	18362500	18600000	98.68	85.67	4.08	10.25	26900.39	50236.43	145.25
	sd	4361134	4385941	1.43	12.55	3.85	9.87	4873.64	5770.66	75.35
	n	12	12	12	12	12	12	12	12	12
	HighConc									
	mean	19287500	19637500	98.35	80.67	5.33	14.00	25639.85	48975.89	190.33
	sd	5198104	5389178	1.54	8.11	4.36	8.82	2394.74	3666.33	84.71
	n	12	12	12	12	12	12	12	12	12
	PosCont									
	mean	138867188	146039063	95.21	34.88	5.88	59.25	22702.44	45606.20	6071.60
	sd	21571218	24038774	1.64	15.08	5.38	18.00	6333.83	4828.32	14082.0
	n	8	8	8	8	8	8	7	7	8

TABLE 13  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Period and Duration*  
(averaging over sex and frequency)

	concentration	duration (hr/day)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	1		5
	100 mg/m <sup>3</sup>	4	<b>3,6,9</b>	<b>4</b>
	200 mg/m <sup>3</sup>	1	<b>6</b>	<b>4</b>
	200 mg/m <sup>3</sup>	4	<b>1,2,6,9</b>	<b>4</b>
	positive control		<b>1,2,6,9</b>	<b>4</b>
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	1		
	100 mg/m <sup>3</sup>	4	1,2,6	
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4	<b>1,2,6</b>	4,8
	positive control		<b>1,2,6,9</b>	<b>3,4,8</b>

*Key for significant effects*

1 = TOTVCELL, 2 = TOTCELL, 3 = PERVCELL,  
4 = MONO, 5 = LYMPH, 6 = NEUT, 7 = PHAGO,  
8 = STDPHAGO, 9 = PROTEIN

normal text:  $p < .05$ , **bold text**:  $p < .01$



TABLE 14  
L06234 Pulmonary Lavage Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(averaging over sex and frequency)

PERIOD	GROUP	DURATION	TOTVCELL	TOTCELL	PERVCELL	MONO	LYMPH	NEUT	PHAGO	STDPHAGO	PROTEIN		
Post-Exp	Control	4 Hr/Day	mean	14920313	15675000	95.00	97.06	1.31	1.63	31959.39	50000.00	63.44	
			sd	6708957	6921055	2.91	3.73	1.66	3.18	4399.50	2041.90	55.61	
			n	16	16	16	16	16	16	16	16	16	
		LowConc	1 Hr/Day	mean	12462500	13025000	95.77	98.33	0.00	1.67	32871.83	48968.77	34.40
				sd	1884526	2023981	2.87	3.20	0.00	3.20	6289.16	2655.11	21.74
				n	6	6	6	6	6	6	6	6	6
			4 Hr/Day	mean	16637500	16933333	98.33	79.50	2.00	18.50	29197.05	49181.33	130.77
				sd	1812716	1965558	1.53	11.57	1.90	10.39	1816.98	1164.17	51.76
				n	6	6	6	6	6	6	6	6	6
	HighConc	1 Hr/Day	mean	12929167	13329167	97.08	84.67	1.83	13.50	30201.81	50186.10	109.42	
			sd	2377678	2491005	1.71	7.69	1.33	7.71	2297.93	1834.64	61.71	
			n	6	6	6	6	6	6	6	6	6	
			4 Hr/Day	mean	22012500	22600000	97.39	69.67	.17	30.17	30713.05	46809.99	128.98
				sd	1046512	878066	2.28	14.35	.41	14.43	6965.43	2551.67	75.18
				n	6	6	6	6	6	6	6	6	6
		PosCont	4 Hr/Day	mean	82171875	87187500	94.42	36.88	3.00	60.13	29945.19	47985.80	504.70
				sd	19864321	21329840	2.86	7.49	2.83	9.06	6908.64	6701.35	138.97
				n	8	8	8	8	8	8	8	8	8

TABLE 14  
L06234 Pulmonary Lavage Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(averaging over sex and frequency)

PERIOD	GROUP	DURATION	TOTVCELL	TOTCELL	PERVCELL	MONO	LYMPH	NEUT	PHAGO	STDPHAGO	PROTEIN
Post-Rec	Control	4 Hr/Day									
		mean	14578125	14728125	99.05	88.56	3.81	7.63	26663.96	50000.00	112.49
		sd	2943961	3025501	1.34	9.97	3.08	7.46	6250.00	4766.88	44.77
		n	16	16	16	16	16	16	16	16	16
	LowConc	1 Hr/Day									
		mean	17262500	17600000	98.12	92.33	2.33	5.33	27320.80	46905.17	88.88
		sd	5304238	5424666	1.84	10.46	4.41	8.19	4347.25	3139.08	51.01
		n	6	6	6	6	6	6	6	6	6
		4 Hr/Day									
		mean	19462500	19600000	99.25	79.00	5.83	15.17	26479.98	53567.69	201.62
		sd	3286934	3239329	.60	11.42	2.40	9.45	5738.68	6064.15	47.56
		n	6	6	6	6	6	6	6	6	6
	HighConc	1 Hr/Day									
		mean	17525000	17737500	98.85	84.83	6.67	8.50	24995.45	52083.16	193.10
		sd	3956608	4036854	.72	7.36	5.09	6.16	2533.59	1894.64	102.05
		n	6	6	6	6	6	6	6	6	6
		4 Hr/Day									
		mean	21050000	21537500	97.85	76.50	4.00	19.50	26284.25	45868.62	187.55
		sd	6027811	6239787	2.03	6.98	3.41	7.79	2280.52	1676.43	73.17
		n	6	6	6	6	6	6	6	6	6
PosCont	4 Hr/Day	mean	138867188	146039063	95.21	34.88	5.88	59.25	22702.44	45606.20	6071.60
		sd	21571218	24038774	1.64	15.08	5.38	18.00	6333.83	4828.32	14082.0
		n	8	8	8	8	8	8	7	7	8

TABLE 15  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Period and Frequency*  
(averaging over sex and duration)

	concentration	frequency (exp/week)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	2		
	100 mg/m <sup>3</sup>	4	<b>6</b>	<b>4</b>
	200 mg/m <sup>3</sup>	2	<b>6</b>	<b>4</b>
	200 mg/m <sup>3</sup>	4	<b>6,9</b>	<b>4</b>
	positive control		<b>1,2,6,9</b>	<b>4</b>
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	2		
	100 mg/m <sup>3</sup>	4	1,2	
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4	1,2	
	positive control		<b>1,2,6,9</b>	<b>3,4</b>

*Key for significant effects*

1 = TOTVCELL, 2 = TOTCELL, 3 = PERVCELL,  
4 = MONO, 5 = LYMPH, 6 = NEUT, 7 = PHAGO,  
8 = STDPHAGO, 9 = PROTEIN

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 16  
L06234 Pulmonary Lavage Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(averaging over sex and duration)

PERIOD	GROUP	Frequency	TOTVCELL	TOTCELL	PERVCELL	MONO	LYMPH	NEUT	PHAGO	STDPHAGO	PROTEIN
Post-Exp	Control	4 / week									
		mean	14920313	15675000	95.00	97.06	1.31	1.63	31959.39	50000.00	63.44
		sd	6708957	6921055	2.91	3.73	1.66	3.18	4399.50	2041.90	55.61
		n	16	16	16	16	16	16	16	16	16
	LowConc	2 / week									
		mean	13512500	13875000	97.14	92.67	1.17	6.17	29036.24	48699.66	70.98
		sd	2804806	2655042	2.12	11.38	1.83	9.56	1901.37	1241.95	47.68
		n	6	6	6	6	6	6	6	6	6
		4 / week									
		mean	15587500	16083333	96.97	85.17	.83	14.00	33032.64	49450.44	94.18
		sd	2616379	2673886	3.17	13.92	1.60	12.65	6144.87	2559.56	78.28
		n	6	6	6	6	6	6	6	6	6
	HighConc	2 / week									
		mean	17041667	17391667	98.03	85.83	1.00	13.17	32909.08	49326.89	91.67
		sd	5479637	5594521	1.60	9.54	1.10	9.87	4873.89	2347.94	61.18
		n	6	6	6	6	6	6	6	6	6
		4 / week									
		mean	17900000	18537500	96.45	68.50	1.00	30.50	28005.78	47669.20	146.73
		sd	5077819	5143801	2.02	11.34	1.55	12.52	3971.68	3090.98	64.23
		n	6	6	6	6	6	6	6	6	6
	PosCont	4 / week									
		mean	82171875	87187500	94.42	36.88	3.00	60.13	29945.19	47985.80	504.70
		sd	19864321	21329840	2.86	7.49	2.83	9.06	6908.64	6701.35	138.97
		n	8	8	8	8	8	8	8	8	8

TABLE 16  
L06234 Pulmonary Lavage Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(averaging over sex and duration)

PERIOD	GROUP	Frequency	TOTVCELL	TOTCELL	PERVCELL	MONO	LYMPH	NEUT	PHAGO	STDPHAGO	PROTEIN
Post-Rec	Control	4 / week									
		mean	14578125	14728125	99.05	88.56	3.81	7.63	26663.96	50000.00	112.49
		sd	2943961	3025501	1.34	9.97	3.08	7.46	6250.00	4766.88	44.77
		n	16	16	16	16	16	16	16	16	16
	LowConc	2 / week									
		mean	18037500	18212500	98.86	91.33	2.83	5.83	29517.75	52516.72	121.63
		sd	5896116	5851042	1.40	11.06	2.79	8.45	5634.32	6763.97	82.35
		n	6	6	6	6	6	6	6	6	6
		4 / week									
		mean	18687500	18987500	98.51	80.00	5.33	14.67	24283.02	47956.14	168.87
		sd	2612506	2779422	1.57	12.13	4.59	9.81	2016.96	3876.93	66.09
		n	6	6	6	6	6	6	6	6	6
	HighConc	2 / week									
		mean	18075000	18287500	98.79	81.00	5.67	13.33	25927.81	49600.92	208.12
		sd	3877145	3874944	.80	5.93	3.88	7.31	1885.76	4363.51	88.82
		n	6	6	6	6	6	6	6	6	6
		4 / week									
		mean	20500000	20987500	97.91	80.33	5.00	14.67	25351.88	48350.86	172.53
		sd	6394060	6671277	2.03	10.44	5.14	10.80	2976.81	3097.51	84.49
		n	6	6	6	6	6	6	6	6	6
PosCont	4 / week	mean	138867188	146039063	95.21	34.88	5.88	59.25	22702.44	45606.20	6071.60
		sd	21571218	24038774	1.64	15.08	5.38	18.00	6333.83	4828.32	14082.0
		n	8	8	8	8	8	8	7	7	8

TABLE 17  
Significant Differences for Exposed Animals as Compared to Filtered-Air Controls  
*Broken Down by Frequency and Duration*  
(averaging over exposure period and sex)

concentration	frequency (exp/week)	duration (hr/day)	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1	<b>4</b>	6
100 mg/m <sup>3</sup>	2	4	1,3, <b>6</b>	4
100 mg/m <sup>3</sup>	4	1		
100 mg/m <sup>3</sup>	4	4	<b>6,9</b>	4
200 mg/m <sup>3</sup>	2	1	<b>6,9</b>	4
200 mg/m <sup>3</sup>	2	4	1,2, <b>6</b>	4
200 mg/m <sup>3</sup>	4	1	<b>6</b>	4
200 mg/m <sup>3</sup>	4	4	1,2, <b>6,9</b>	4,8
positive control			1,2, <b>6,9</b>	3,4,8

*Key for significant effects*

1 = TOTVCELL, 2 = TOTCELL, 3 = PERVCELL,  
4 = MONO, 5 = LYMPH, 6 = NEUT, 7 = PHAGO,  
8 = STDPHAGO, 9 = PROTEIN

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 18  
L06234 Pulmonary Lavage Analysis of All Animals  
Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
(averaging over exposure period and sex)

GROUP	FREQUENCY	DURATION	TOTVCELL	TOTCELL	PERVCELL	MONO	LYMPH	NEUT	PHAGO	STDPHAGO	PROTEIN
Control	4 / week	4 Hr/Day									
		mean	14749219	15201563	97.02	92.81	2.56	4.63	29311.67	50000.00	87.97
		sd	5099310	5276217	3.03	8.57	2.75	6.41	5958.47	3607.28	55.56
		n	32	32	32	32	32	32	32	32	32
LowConc	2 / week	1 Hr/Day									
		mean	13075000	13500000	96.65	99.50	.50	0.00	29155.33	48506.18	48.92
		sd	4840300	4835494	1.82	1.22	1.22	0.00	2727.17	2170.25	20.85
		n	6	6	6	6	6	6	6	6	6
		4 Hr/Day									
		mean	18475000	18587500	99.34	84.50	3.50	12.00	29398.66	52710.21	143.70
		sd	3740588	3696037	.74	10.78	2.43	8.74	5294.05	6381.33	69.06
		n	6	6	6	6	6	6	6	6	6
	4 / week	1 Hr/Day									
		mean	16650000	17125000	97.24	91.17	1.83	7.00	31037.31	47367.76	74.37
		sd	3796512	3849091	3.38	9.91	4.49	7.48	8208.40	3737.77	63.50
		n	6	6	6	6	6	6	6	6	6
		4 Hr/Day									
		mean	17625000	17945833	98.24	74.00	4.33	21.67	26278.36	50038.82	188.68
		sd	2110154	2171544	1.39	9.06	3.44	8.45	2606.65	2161.03	44.36
		n	6	6	6	6	6	6	6	6	6

TABLE 18  
L06234 Pulmonary Lavage Analysis of All Animals  
Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
(averaging over exposure period and sex)

GROUP	FREQUENCY	DURATION	TOTVCELL	TOTCELL	PERVCELL	MONO	LYMPH	NEUT	PHAGO	STDPHAGO	PROTEIN	
HighConc	2 / week	1 Hr/Day	mean	15991667	16254167	98.38	86.33	4.17	9.50	27959.07	51719.53	193.97
			sd	4702863	4776935	1.26	6.09	3.60	5.54	2416.85	2804.84	111.75
			n	6	6	6	6	6	6	6	6	6
		4 Hr/Day	mean	19125000	19425000	98.43	80.50	2.50	17.00	30877.82	47208.28	105.82
			sd	4206156	4236006	1.41	9.09	3.83	9.30	6752.86	2126.08	51.16
			n	6	6	6	6	6	6	6	6	6
	4 / week	1 Hr/Day	mean	14462500	14812500	97.55	83.17	4.33	12.50	27238.18	50549.73	108.55
			sd	3234376	3177292	1.82	8.38	5.35	8.73	4670.15	653.22	39.48
			n	6	6	6	6	6	6	6	6	6
		4 Hr/Day	mean	23937500	24712500	96.81	65.67	1.67	32.67	26119.48	45470.33	210.72
			sd	2529365	2380113	2.42	8.24	2.34	10.17	2510.04	1876.34	62.00
			n	6	6	6	6	6	6	6	6	6
PosCont	4 / week	4 Hr/Day	mean	110519531	116613281	94.81	35.87	4.44	59.69	26565.24	46875.32	3288.15
			sd	35474681	37491153	2.29	11.55	4.41	13.77	7412.33	5827.11	10040.6
			n	16	16	16	16	16	16	15	15	16



PART TWO

APPENDIX

SECTION H. PULMONARY FUNCTION

IIT RESEARCH INSTITUTE

SA/H-1

## 1 L06234 Statistical Report - Pulmonary Function

Multivariate analysis of variance models (Bock, 1975) were used to analyze the pulmonary function measures. These variables are organized into the following 4 subtests (1) VCPV, CCHORD, (2) FVC, FEV100, FEF50, (3) FOB, VT, PES, RL, CDYN, (4) TLC, DLCO, VC. Each of these subsets of measurements were analyzed separately. In addition, 15 other variables were examined for exploratory purposes. These exploratory variables are CPK, FEV50, FEV200, FEV400, PEXF, VPEXF, MMEXF, FEF25, FEF75, VE, TI, TE, VEMAX, VIMAX, and RV. Tables summarizing the significant main effects and interactions, with and without covariate adjustment for body weight are presented throughout the report, however, only the primary variables from the 4 subtests are discussed in the body of the report so that statistical inference regarding the effects of treatment is restricted to the primary measures of interest.

Prior to analysis, log transformation of the data was performed on these variables to better approximate the normality assumption of the statistical model. The effects of five factors were examined in these analyses: Sex (male or female), Period (exposure or recovery), concentration (control,  $100 \text{ mg/m}^3$ ,  $200 \text{ mg/m}^3$ , or positive control at  $200 \text{ mg/m}^3$ ), duration (1 or 4 hours per day) and frequency (2 or 4 exposures per week). These analyses were performed with and without covariate adjustment for the effects of body weight. The animals were allocated to the resulting cells of the design in a fractional factorial manner (Winer, 1971) which would allow tests of all two-way interactions to be made, with the exception that only the two dosed animal groups were allocated to the cells with varying duration and frequency. As a result, two sets of analyses were performed on the data. In the first, only the two dosed groups were examined using a multivariate analysis of variance model which included all five factors and all two-way interactions.

The second set of analyses concentrated on examining the differences between the dosed and control animals. First, a one-factor multivariate analysis of variance was performed on each of the 4 pulmonary function subtests for the exposure and recovery animals separately, with concentration as the grouping factor, augmented by simple contrasts. This allowed a statistical comparison to be made between each group ( $100 \text{ mg/m}^3$ ,  $200 \text{ mg/m}^3$ , and positive control at  $200 \text{ mg/m}^3$ ) and the control group for the exposure and recovery animals separately. In order to examine the consistency of any group differences between the levels of duration, a second multivariate analysis of variance was performed, again, separately for the exposure and recovery animals. In this second analysis, the low and high dose animals were further grouped depending on their duration of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the duration subgroups. A third multivariate analysis of variance was performed for the exposure and recovery animals in order to examine the consistency of any group differences between the levels of frequency. In this analysis, the low and high dose animals were further grouped depending on their frequency of exposure. In this way, again using a one-factor multivariate analysis of variance augmented by simple contrasts, we could examine whether a difference between the dosed and control groups was consistent across the frequency subgroups. A final multivariate analysis of variance was performed, combining the exposure and recovery animals, to examine the consistency of any group differences between the levels of both frequency and duration. In this analysis, the low and high dose animals were further grouped depending on both their duration and frequency of exposure. The one-way multivariate analysis of variance was augmented with simple contrasts to examine the possibility of a group by duration by frequency interaction.

## 1.1 Analysis of Dosed Animals - Fractional Factorial Analysis

The multivariate analysis of variance yielded significant interaction effects of duration by frequency (subtest 1 with and without adjustment for body weight), concentration by frequency (subtest 3 with and without adjustment for body weight), concentration by duration (subtests 1 and 3 with and without adjustment for body weight), sex by duration (subtest 2 with and without adjustment for body weight), and period by duration (subtest 4 only after adjusting for body weight). A significant main effect of sex was observed for all 4 subtests, but in all cases it was nonsignificant following adjustment for body weight. Finally, a main effect of period (*i.e.*, treatment vs recovery sacrifice) was found for subtests 2-4, however, the effect for subtest 2 was not significant following body weight adjustment. All other main effects (*e.g.*, duration, frequency, and concentration) and two way interactions were observed to be non-significant by the multivariate test.

The duration by frequency interaction was observed to be significant for VCPV and CCHORD. The observed means for the subgroups defined by duration and frequency, listed in Table 1, reveal that for the low frequency, the high duration produced increased levels for both measures, whereas the reverse was true for the high frequency.

The concentration by frequency interaction was observed to be significant for VT, RL, and CDYN. The observed means for the subgroups defined by concentration and frequency, listed in Table 7, reveal that for the low dose, the high frequency produced increased levels for all 3 measures, whereas the reverse was true for the high dose. It should be noted, however, that this effect on VT was only found when adjusting for body weight, whereas for the other two variables (RL and CDYN) this effect was observed both with and without body weight adjustment.

The concentration by duration interaction was observed to be significant for VCPV, CCHORD, PES, RL, CDYN, and VT. The observed means for the subgroups defined by concentration and duration, listed in Tables 2 and 8, reveal that for the low dose, the high duration produced increased levels for all VCPV, CCHORD, PES, and RL (and decreased levels on CDYN and VT), whereas the reverse was true for the high dose. It should be noted, however, that this effect on VT was only found without the body weight adjustment, whereas for the other variables this effect was observed both with and without body weight adjustment.

The sex by duration interaction was observed to be significant for FVC, and FEV100. The observed means for the subgroups defined by sex and duration, listed in Table 4, reveal that for the males, the high duration produced decreased levels for both measures, whereas the reverse was true for the females.

The period by concentration interaction was observed to be significant for TLC and VC. The observed means for the subgroups defined by period and concentration, listed in Table 11, reveal that at the exposure sacrifice, the high dose produced decreased levels for both measures, whereas the reverse was true following recovery. It should be noted, however, that this effect was only found after adjusting for the effect of body weight.

The main effect of sex was significant for VCPV, CCHORD, FVC, FEV100, FOB, VT, RL, CDYN, TLC, DLCO, and VC (see Tables 3, 5, 9, and 12). Females were decreased relative to males on all variables with the exception of RL, where females were elevated relative to males. None of these differences were significant following adjustment for body weight.

The main effect of period was significant for FVC, FEV100, VT, PES, CDYN, FOB, TLC, DLCO, and VC (see Tables 6, 10, and 13). At the treatment sacrifice, FVC, FEV100, PES, TLC, and VC were decreased relative to recovery, whereas VT, CDYN, FOB, and DLCO were increased relative to recovery. The effects for FVC, FEV100, TLC, and VC were only significant for the unadjusted data. The effects for FOB and PES were only significant for the body weight adjusted data. The effects for VT, CDYN, and DLCO were significant for both adjusted and unadjusted

data.

Tables 14-25 present summary statistics for all main effects and interactions that were statistically significant for the 15 exploratory measures. Significance testing was based solely on the univariate F-statistics, and as such, some of these effects may indeed be consistent with chance expectations.

## **1.2 Analysis of All Animals - Comparison to Controls**

### **1.2.1 Subtest 1 - VCPV and CCHORD**

In the first set of analyses, concentration differences were examined separately for the exposure and recovery animals. The significant differences that were observed are listed in Table 26, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 27. No significant concentration versus control differences were observed at either the exposure or recovery period, when averaging over sex, frequency, and duration.

In the second set of analyses, concentration differences were again examined separately for exposure and recovery animals. However, in order to also examine the effect of duration, the low and high dose animals were further divided into subgroups depending on their duration level. The significant differences that were observed for the exposure and recovery animals are listed in Table 28, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 29. Inspection of Table 28 reveals that the low dose low duration and high dose high duration exhibited significantly decreased VCPV and CCHORD levels at the exposure sacrifice, following adjustment for the effect of body weight. These two effects were not seen following recovery or in the unadjusted data.

In the third set of analyses, concentration differences were again examined separately for exposure and recovery animals, however, in order to also examine the effect of frequency, the low and high dose animals were further divided into subgroups depending on their frequency level. The significant differences that were observed for the exposure and recovery animals are listed in Table 30, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 31. No significant differences were observed.

In the fourth and final set of analyses, concentration differences were examined combining the exposure and recovery animals, in order to examine the interaction effect of duration and frequency. Here, the low and high dose animals were further divided into subgroups depending on both their frequency and duration level. The significant differences that were observed are listed in Table 32, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 33. Inspection of Table 32 reveals that VCPV and CCHORD were significantly decreased for the low dose, low frequency, low duration group relative to controls and for the high dose, high frequency, high duration group relative to controls. In addition, CCHORD levels were significantly decreased in positive controls and low dose, high frequency, high duration animals relative to controls. All of these differences were only significant in the body weight adjusted data.

### **1.2.2 Subtest 2 - FVC, FEV100, and FEF50**

In the first set of analyses, concentration differences were examined separately for the exposure and recovery animals. The significant differences that were observed are listed in Table 34, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 35. Inspection of Table 34 reveals that high dose exposure animals and both low dose and positive control recovery animals exhibited significantly decreased FVC levels relative to controls. These effects were only significant following adjustment for body weight.

In the second set of analyses, concentration differences were again examined separately for exposure and recovery animals. However, in order to also examine the effect of duration, the low and high dose animals were further divided into subgroups depending on their duration level. The significant differences that were observed for the exposure and recovery animals are listed in Table 36, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 37. Inspection of Table 36 reveals no significant treatment related effects.

In the third set of analyses, concentration differences were again examined separately for exposure and recovery animals, however, in order to also examine the effect of frequency, the low and high dose animals were further divided into subgroups depending on their frequency level. The significant differences that were observed for the exposure and recovery animals are listed in Table 38, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 39. During the exposure period, FVC and FEV100 were significantly decreased in low dose, high frequency animals and high dose low frequency animals relative to controls. Following recovery, low dose low frequency and positive control animals exhibited decreased FVC levels relative to controls. These effects were only significant following adjustment for body weight.

In the fourth and final set of analyses, concentration differences were examined combining the exposure and recovery animals, in order to examine the interaction effect of duration and frequency. Here, the low and high dose animals were further divided into subgroups depending on both their frequency and duration level. The significant differences that were observed are listed in Table 40, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 41. Inspection of Table 40 reveals no significant treatment related effects.

### 1.2.3 Subtest 3 - FOB, VT, PES, RL, CDYN

In the first set of analyses, concentration differences were examined separately for the exposure and recovery animals. The significant differences that were observed are listed in Table 42, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 43. Inspection of Table 42 reveals that exposure animals had increased FOB levels relative to controls following body weight adjustment for low and high dose, and regardless of body weight for positive controls. Following recovery, the effect on FOB in positive controls remained, and in addition positive controls exhibited decreased VT, RL, and CDYN levels relative to controls. In addition, RL was decreased in the low dose recovery animals as well. These effects in recovery animals were observed for both adjusted and unadjusted data.

In the second set of analyses, concentration differences were again examined separately for exposure and recovery animals. However, in order to also examine the effect of duration, the low and high dose animals were further divided into subgroups depending on their duration level. The significant differences that were observed for the exposure and recovery animals are listed in Table 44, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 45. Inspection of Table 44 reveals that for exposure animals, FOB was increased in low dose and high dose high duration animals following adjustment for body weight only, and in positive controls regardless of body weight. Following the recovery period, the effect on positive controls remained constant, and in addition, significant decreases were observed for VT, RL and CDYN for positive controls, RL for high dose high duration animals, and PES and RL in low dose low duration animals relative to controls. With the exception of the single effect on VT, which was only observed following adjustment for body weight, all other effects were independent of body weight.

In the third set of analyses, concentration differences were again examined separately for exposure and recovery animals, however, in order to also examine the effect of frequency, the low and high dose animals were further divided into subgroups depending on their frequency level. The

significant differences that were observed for the exposure and recovery animals are listed in Table 46, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 47. During the exposure period, FOB levels were significantly increased in low dose, low frequency animals (adjusted data only) and positive controls. Following recovery, low dose low frequency animals had decreased VT and RL levels (adjusted data only), and positive controls still had increased FOB levels and decreased VT, RL (adjusted data only), and CDYN levels relative to controls.

In the fourth and final set of analyses, concentration differences were examined combining the exposure and recovery animals, in order to examine the interaction effect of duration and frequency. Here, the low and high dose animals were further divided into subgroups depending on both their frequency and duration level. The significant differences that were observed are listed in Table 48, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 49. Inspection of Table 48 reveals that RL was significantly decreased in low dose low frequency low duration animals and high dose high frequency high duration animals. In addition, positive controls exhibited increased FOB levels relative to controls. These effects were significant regardless of body weight.

#### **1.2.4 Subtest 4 - TLC, DLCO, and VC**

In the first set of analyses, concentration differences were examined separately for the exposure and recovery animals. The significant differences that were observed are listed in Table 50, while the concentration means, standard deviations, and sample sizes for these subsamples are given in Table 51. Inspection of Table 50 reveals that exposure animals had decreased TLC and VC levels relative to controls following body weight adjustment for low dose, high dose, and positive controls. In addition, positive controls also exhibited decreased DLCO following adjustment for body weight. Following recovery, low dose animals had decreased DLCO and VC levels (adjusted data only), and positive controls had decreased DLCO regardless of body weight.

In the second set of analyses, concentration differences were again examined separately for exposure and recovery animals. However, in order to also examine the effect of duration, the low and high dose animals were further divided into subgroups depending on their duration level. The significant differences that were observed for the exposure and recovery animals are listed in Table 52, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 53. Inspection of Table 52 reveals that for exposure animals, TLC and VC were decreased in low dose low duration animals, high dose low and high duration animals, and positive controls. In addition, positive controls also exhibited decreased DLCO levels relative to controls. All of these effects were only found following adjustment for body weight. Following the recovery period, all but high dose low duration animals had decreased DLCO, and this effect was only found after adjustment for body weight, except in positive controls.

In the third set of analyses, concentration differences were again examined separately for exposure and recovery animals, however, in order to also examine the effect of frequency, the low and high dose animals were further divided into subgroups depending on their frequency level. The significant differences that were observed for the exposure and recovery animals are listed in Table 54, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 55. During the exposure period, TLC and VC levels were significantly decreased in all treated and positive control groups (adjusted data only). In addition, DLCO levels were also decreased in high dose high frequency and positive controls relative controls (adjusted data only). Following recovery, low dose low frequency animals had decreased TLC and VC levels (adjusted data only), high dose high frequency animals had decreased TLC (adjusted data only), and positive controls

had decreased DLCO levels regardless of body weight.

In the fourth and final set of analyses, concentration differences were examined combining the exposure and recovery animals, in order to examine the interaction effect of duration and frequency. Here, the low and high dose animals were further divided into subgroups depending on both their frequency and duration level. The significant differences that were observed are listed in Table 56, while the group means, standard deviations, and sample sizes for these subsamples are given in Table 57. Inspection of Table 56 reveals that TLC and VC were significantly decreased in low dose low frequency low duration animals, low dose high frequency low duration animals, low dose high frequency high duration animals, high dose low frequency high duration animals, high dose high frequency high duration animals, and positive controls. In addition, high dose high frequency low duration animals had decreased VC levels only. With the exception of the effect on DLCO in positive controls, all other significant differences were only observed after covariate adjustment for body weight.

#### 1.2.5 Exploratory Measures

Results and summary statistics for the 15 exploratory measures, which parallel the hypothesis testing measures, are presented in Table 58-65. As in the four subtests, significant differences for the exploratory measures, were generally only observed following the statistical adjustment for body weight.

### 1.3 Summary

Overall, there were very few treatment related effects on the pulmonary function variables, and where they did occur, it was generally after adjustment for body weight only. No clear effects of concentration, duration, or frequency were observed, and the absence of significant main effects and the few scattered two-way interactions supports this conclusion. In terms of overall differences from control, some reasonably consistent decreases in RL following the recovery period were observed, and these effects appeared to be independent of body weight. Conversely, TLC and VC appeared to be decreased in exposure animals, but these effects only appeared after adjusting for body weight.

## References

- [1] Bock, R. D. *Multivariate statistical methods in behavioral research*. New York: McGraw-Hill, 1975.
- [2] Winer, B. J. *Statistical principles in experimental design*, 2nd edition. New York: McGraw-Hill, 1971.

TABLE 1  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Frequency by Duration Subgroups  
 (Averaging over Concentration, Period, and Sex)  
 (variables with significant univariate F-test results)

FREQUENCY	DURATION	VCPV	CCHORD	
2 / week	1 Hr/Day			
	mean	7.67	.48	
	sd	1.23	.08	
	n	12	12	
	4 Hr/Day			
	mean	8.45	.53	
	sd	.79	.05	
	n	12	12	
	4 / week	1 Hr/Day		
		mean	8.39	.53
sd		1.10	.08	
n		11	11	
4 Hr/Day				
mean		7.95	.49	
sd		1.31	.10	
n		12	12	
OVERALL		mean	8.11	.51
		sd	1.14	.08
	n	47	47	



TABLE 2  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Duration Subgroups  
 (Averaging over Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	DURATION	VCPV	CCHORD
LowConc	1 Hr/Day		
		mean	.48
		sd	.07
		n	12
	4 Hr/Day		
		mean	.52
		sd	.10
		n	12
HighConc	1 Hr/Day		
		mean	.53
		sd	.08
		n	11
	4 Hr/Day		
		mean	.50
		sd	.06
		n	12
OVERALL	mean	8.11	.51
	sd	1.14	.08
	n	47	47

TABLE 3  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	VCPV	CCHORD
Male		
mean	8.87	.56
sd	.71	.05
n	24	24
Female		
mean	7.32	.45
sd	.95	.07
n	23	23
OVERALL mean	8.11	.51
sd	1.14	.08
n	47	47

TABLE 4  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Duration Subgroups  
 (Averaging over Concentration, Frequency, and Period)  
 (variables with significant univariate F-test results)

SEX	DURATION	FVC	FEV100
Male	1 Hr/Day		
		mean	8.66
		sd	.65
		n	12
	4 Hr/Day		
		mean	8.31
		sd	.68
		n	12
Female	1 Hr/Day		
		mean	6.62
		sd	.45
		n	11
	4 Hr/Day		
		mean	7.32
		sd	.50
		n	12
OVERALL	mean	7.75	5.83
	sd	.98	.71
	n	47	47

TABLE 5  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX		FVC	FEV100
Male			
	mean	8.48	6.19
	sd	.67	.72
	n	24	24
Female			
	mean	6.99	5.45
	sd	.59	.48
	n	23	23
OVERALL			
	mean	7.75	5.83
	sd	.98	.71
	n	47	47

TABLE 6  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	FVC	FEV100
Post-Exp		
mean	7.53	5.50
sd	.84	.57
n	24	24
Post-Rec		
mean	7.98	6.17
sd	1.03	.69
n	23	23
OVERALL mean	7.75	5.83
sd	.98	.71
n	47	47

TABLE 7  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Frequency Subgroups  
 (Averaging over Duration, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	FREQUENCY	RL	CDYN	VT
LowConc	2 / week			
	mean	.11	.22	1.38
	sd	.06	.06	.37
	n	11	12	12
	4 / week			
	mean	.15	.26	1.51
	sd	.05	.05	.28
	n	11	12	12
	HighConc	2 / week		
mean		.14	.25	1.47
sd		.03	.07	.27
n		11	12	12
4 / week				
mean		.11	.24	1.43
sd		.05	.05	.18
n		10	11	11
OVERALL		mean	.13	.24
	sd	.05	.06	.28
	n	43	47	47

TABLE 8  
 LC6234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Duration Subgroups  
 (Averaging over Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRAT	DURATION	VT	PES	RL	CDYN
LowConc	1 Hr/Day				
	mean	1.47	6.45	.09	.26
	sd	.37	1.01	.05	.07
	n	12	12	10	12
	4 Hr/Day				
	mean	1.41	7.73	.16	.22
	sd	.30	1.00	.04	.04
	n	12	12	12	12
	HighConc	1 Hr/Day			
mean		1.41	7.00	.14	.24
sd		.16	1.33	.04	.06
n		11	11	11	11
4 Hr/Day					
mean		1.48	6.66	.12	.26
sd		.28	.90	.05	.06
n		12	12	10	12
OVERALL		mean	1.45	6.96	.13
	sd	.28	1.14	.05	.06
	n	47	47	43	47

TABLE 9  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	FOB	VT	RL	CDYN
Male				
mean	106.78	1.63	.11	.27
sd	13.75	.24	.05	.05
n	24	24	24	24
Female				
mean	76.38	1.26	.15	.22
sd	12.94	.17	.05	.05
n	23	23	19	23
OVERALL mean	91.91	1.45	.13	.24
sd	20.26	.28	.05	.06
n	47	47	43	47



TABLE 10  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	VT	CDYN	FOB	PES
Post-Exp				
mean	1.51	.26	92.28	6.66
sd	.32	.06	19.97	1.27
n	24	24	24	24
Post-Rec				
mean	1.38	.23	91.51	7.27
sd	.21	.05	21.00	.93
n	23	23	23	23
OVERALL mean				
sd	1.45	.24	91.91	6.96
n	.28	.06	20.26	1.14
	47	47	47	47

TABLE 11  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period by Concentration Subgroups  
 (Averaging over Frequency, Duration, Period, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	CONCENTRATION	TLC	VC
Post-Exp	LowConc		
		mean	8.87
		sd	.67
		n	11
	HighConc		
		mean	8.39
		sd	.71
		n	11
Post-Rec	LowConc		
		mean	9.16
		sd	1.05
		n	12
	HighConc		
		mean	9.69
		sd	.83
		n	11
OVERALL	mean	9.03	7.86
	sd	1.12	.90
	n	45	45

TABLE 12  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	TLC	DLCO	VC
Male			
mean	9.95	.15	8.59
sd	.70	.02	.61
n	22	22	22
Female			
mean	8.15	.10	7.17
sd	.62	.01	.48
n	23	23	23
OVERALL mean	9.03	.13	7.86
sd	1.12	.03	.90
n	45	45	45

TABLE 13  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	TLC	DLCO	VC
Post-Exp			
mean	8.63	.13	7.50
sd	.91	.03	.69
n	22	22	22
Post-Rec			
mean	9.42	.12	8.21
sd	1.18	.03	.95
n	23	23	23
OVERALL mean	9.03	.13	7.86
sd	1.12	.03	.90
n	45	45	45

TABLE 14  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Frequency by Duration Subgroups  
 (Averaging over Concentration, Period, and Sex)  
 (variables with significant univariate F-test results)

FREQUENCY	DURATION	CPK	VPEXF
2 / week	1 Hr/Day		
		mean	2.44
		sd	.65
		n	12
	4 Hr/Day		
		mean	2.83
		sd	.45
		n	12
4 / week	1 Hr/Day		
		mean	2.44
		sd	.80
		n	11
	4 Hr/Day		
		mean	2.14
		sd	.34
		n	12
OVERALL	mean	.85	2.46
	sd	.13	.62
	n	44	47

TABLE 15  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Frequency Subgroups  
 (Averaging over Duration, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	FREQUENCY	PEXF	FEF25	FEF75	VE
LowConc	2 / week				
	mean	91.16	43.43	89.00	127.34
	sd	15.24	5.96	15.25	50.86
	n	12	12	12	12
	4 / week				
	mean	88.53	44.45	85.79	147.09
	sd	21.80	8.15	19.82	56.74
	n	12	12	12	12
HighConc	2 / week				
	mean	81.34	48.00	79.44	141.82
	sd	16.44	8.95	16.24	47.83
	n	12	12	12	12
	4 / week				
	mean	93.07	40.88	91.52	131.94
	sd	16.38	11.42	16.91	38.38
	n	11	11	11	11
OVERALL	mean	88.43	44.26	86.33	137.16
	sd	17.67	8.86	17.19	48.16
	n	47	47	47	47

TABLE 16  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Concentration by Duration Subgroups  
 (Averaging over Frequency, Period, and Sex)  
 (variables with significant univariate F-test results)

CONCENTRATION	DURATION	FEV50	PEXF	MMEXF	FEF75
LowConc	1 Hr/Day				
		mean	2.98	99.86	73.49
		sd	.60	20.22	7.98
		n	12	12	12
	4 Hr/Day				
		mean	2.42	79.82	65.08
		sd	.34	9.15	4.59
		n	12	12	12
HighConc	1 Hr/Day				
		mean	2.54	83.03	66.76
		sd	.52	15.85	13.73
		n	11	11	11
	4 Hr/Day				
		mean	2.71	90.55	70.35
		sd	.63	18.11	8.63
		n	12	12	12
OVERALL	mean	2.67	88.43	68.96	86.33
	sd	.56	17.67	9.49	17.19
	n	47	47	47	47

TABLE 17  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	FREQUENCY	FEF25	VIMAX
Male	2 / week		
		mean	46.41
		sd	7.10
		n	12
	4 / week		
		mean	47.14
		sd	10.18
		n	12
Female	2 / week		
		mean	45.03
		sd	8.69
		n	12
	4 / week		
		mean	37.94
		sd	6.93
		n	11
OVERALL	mean	44.26	10.89
		sd	8.86
		n	47



TABLE 18  
L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
Means, SDs, and Ns for Sex by Duration Subgroups  
(Averaging over Concentration, Frequency, and Period)  
(variables with significant univariate F-test results)

SEX	DURATION	FEV50	FEV200	FEV400	PEXF	FEF75	TE	RV		
Male	1 Hr/Day	mean	2.99	8.19	8.56	100.49	97.47	.28	1.46	
		sd	.65	.64	.64	21.31	19.79	.06	.22	
		n	12	12	12	12	12	12	10	
	4 Hr/Day	mean	2.55	7.83	8.19	84.48	82.90	.32	1.29	
		sd	.64	.60	.64	19.00	19.12	.07	.13	
		n	12	12	12	12	12	12	12	
	Female	1 Hr/Day	mean	2.53	6.33	6.56	82.34	80.67	.53	.95
			sd	.43	.38	.42	13.22	13.23	.12	.12
			n	11	11	11	11	11	11	11
4 Hr/Day		mean	2.58	6.90	7.21	85.90	83.81	.48	1.02	
		sd	.39	.45	.45	10.60	11.51	.11	.22	
		n	12	12	12	12	12	12	12	
OVERALL	mean	2.67	7.33	7.65	88.43	86.33	.40	1.17		
	sd	.56	.90	.95	17.67	17.19	.14	.27		
	n	47	47	47	47	47	47	45		

TABLE 19  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex by Concentration Subgroups  
 (Averaging over Frequency, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	CONCENTRATION	VIMAX
Male	LowConc	
		mean 12.63
		sd 1.73
		n 12
	HighConc	
		mean 11.54
		sd 1.90
		n 12
Female	LowConc	
		mean 9.27
		sd 1.05
		n 12
	HighConc	
		mean 10.03
		sd 1.69
		n 11
OVERALL		mean 10.89
		sd 2.06
		n 47

TABLE 20  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period by Frequency Subgroups  
 (Averaging over Concentration, Duration, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	FREQUENCY	MMEXF
Post-Exp	2 / week	
		mean 64.49
		sd 7.59
		n 12
	4 / week	
		mean 64.02
		sd 5.61
		n 12
Post-Rec	2 / week	
		mean 76.70
		sd 8.55
		n 12
	4 / week	
		mean 70.80
		sd 10.34
		n 11
OVERALL	mean	68.96
	sd	9.49
	n	47

TABLE 21  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period by Concentration Subgroups  
 (Averaging over Frequency, Duration, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	CONCENTRATION	RV
Post-Exp	LowConc	
		mean 1.21
		sd .22
		n 11
	HighConc	
		mean 1.05
		sd .28
		n 11
Post-Rec	LowConc	
		mean 1.14
		sd .26
		n 12
	HighConc	
		mean 1.28
		sd .28
		n 11
OVERALL	mean	1.17
	sd	.27
	n	45

TABLE 22  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period by Sex Subgroups  
 (Averaging over Concentration, Frequency, and Duration)  
 (variables with significant univariate F-test results)

PERIOD	SEX	VEMAX	VE
Post-Exp	Male		
		mean	189.43
		sd	29.23
		n	12
	Female		
		mean	98.89
		sd	18.57
		n	12
Post-Rec	Male		
		mean	160.69
		sd	35.57
		n	12
	Female		
		mean	96.21
		sd	20.28
		n	11
OVERALL	mean	-9.08	137.16
	sd	1.81	48.16
	n	47	47

TABLE 23  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Frequency Subgroups  
 (Averaging over Concentration, Duration, Period, and Sex)  
 (variables with significant univariate F-test results)

FREQUENCY	VPEXF	MMEXF
2 / week		
mean	2.63	70.59
sd	.58	10.07
n	24	24
4 / week		
mean	2.28	67.26
sd	.61	8.73
n	23	23
OVERALL mean	2.46	68.96
sd	.62	9.49
n	47	47

TABLE 24  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Sex Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Period)  
 (variables with significant univariate F-test results)

SEX	CPK	FEV200	FEV400	FEF25	VE	TE	VEMAX	VIMAX	RV	MMEXF
Male										
mean	.94	8.01	8.37	46.78	175.06	.30	-10.35	12.09	1.37	71.22
sd	.08	.63	.65	8.59	35.06	.07	1.47	1.86	.19	8.59
n	21	24	24	24	24	24	24	24	22	24
Female										
mean	.76	6.63	6.90	41.64	97.61	.50	-7.76	9.63	.99	66.61
sd	.10	.50	.54	8.53	19.01	.12	.98	1.42	.18	9.99
n	23	23	23	23	23	23	23	23	23	23
OVERALL mean	.85	7.33	7.65	44.26	137.16	.40	-9.08	10.89	1.17	68.96
sd	.13	.90	.95	8.86	48.16	.14	1.81	2.06	.27	9.49
n	44	47	47	47	47	47	47	47	45	47

TABLE 25  
 L06234 Pulmonary Function Analysis of Graphite-Exposed Animals  
 Means, SDs, and Ns for Period Subgroups  
 (Averaging over Concentration, Frequency, Duration, and Sex)  
 (variables with significant univariate F-test results)

PERIOD	FEV50	FEV200	FEV400	PEXF	MMEXF	FEF25	FEF75	VE	VIMAX	VPEXF	TE	VEMAX
Post-Exp												
mean	2.44	7.08	7.43	82.41	64.26	41.19	80.27	144.16	10.28	2.59	.39	-9.24
sd	.52	.75	.81	16.81	6.53	8.59	16.68	52.08	1.80	.70	.12	2.15
n	24	24	24	24	24	24	24	24	24	24	24	24
Post-Rec												
mean	2.90	7.59	7.89	94.71	73.88	47.47	92.65	129.85	11.51	2.33	.41	-8.91
sd	.50	.97	1.05	16.64	9.70	8.11	15.66	43.64	2.15	.50	.16	1.40
n	23	23	23	23	23	23	23	23	23	23	23	23
OVERALL mean	2.67	7.33	7.65	88.43	68.96	44.26	86.33	137.16	10.89	2.46	.40	-9.08
sd	.56	.90	.95	17.67	9.49	8.86	17.19	48.16	2.06	.62	.14	1.81
n	47	47	47	47	47	47	47	47	47	47	47	47



TABLE 26  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period*  
(averaging over sex, frequency and duration)

		significant increases	significant decreases
<i>Post-Exposure</i>	concentration		
	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>		
	positive control		
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>		
	positive control		

*Key for significant effects*

1 = VCPV, 2 = CCHORD

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 27  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration Subgroups  
(Averaging over Sex, Frequency and Duration)

PERIOD	GROUP	VCPV	CCHORD
Post-Exp	Control		
		mean	8.50
		sd	.53
		n	.09
	LowConc		
		mean	8.11
		sd	.50
		n	.06
	HighConc		
		mean	7.84
		sd	.48
		n	.08
	PosCont		
		mean	8.18
		sd	.49
		n	.08
Post-Rec	Control		
		mean	8.72
		sd	.55
		n	.10
	LowConc		
		mean	8.04
		sd	.50
		n	.11
	HighConc		
		mean	8.49
		sd	.54
		n	.06
	PosCont		
		mean	7.81
		sd	.49
		n	.06

TABLE 28  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Duration*  
(averaging over sex and frequency)

	concentration	duration (hr/day)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	1		(1.2)
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4		(1.2)
	positive control			
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	1		
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4		
	positive control			

*Key for significant effects*

1 = VCPV, 2 = CCHORD

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 29  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	VCPV	CCHORD
Post-Exp	Control	4 Hr/Day		
		mean	8.50	.53
		sd	1.45	.09
		n	16	16
	LowConc	1 Hr/Day		
		mean	7.43	.46
		sd	.72	.05
		n	6	6
		4 Hr/Day		
		mean	8.80	.55
		sd	.47	.03
		n	6	6
	HighConc	1 Hr/Day		
		mean	8.26	.51
		sd	1.33	.09
		n	6	6
		4 Hr/Day		
		mean	7.41	.46
		sd	.98	.05
		n	6	6
	PosCont	4 Hr/Day		
		mean	8.18	.49
		sd	1.22	.08
		n	8	8

TABLE 29  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
 (Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	VCPV	CCHORD
Post-Rec	Control	4 Hr/Day		
			mean	.55
			sd	.10
			n	16
		1 Hr/Day		
			mean	.50
			sd	.09
			n	6
	LowConc	4 Hr/Day		
			mean	.50
			sd	.14
			n	6
		1 Hr/Day		
			mean	.55
			sd	.08
			n	5
	HighConc	4 Hr/Day		
			mean	.53
			sd	.05
			n	6
		1 Hr/Day		
			mean	.55
			sd	.08
			n	5
	PosCont	4 Hr/Day		
			mean	.49
			sd	.06
			n	7

TABLE 30  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Frequency*  
(averaging over sex and duration)

	concentration	frequency (exp/week)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	2		
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4		
	positive control			
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	2		
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4		
	positive control			

*Key for significant effects*

1 = VCPV, 2 = CCHORD

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 31  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	VCPV	CCHORD
Post-Exp	Control	4 / week		
		mean	8.50	.53
		sd	1.45	.09
		n	16	16
	LowConc	2 / week		
		mean	8.22	.51
		sd	.32	.04
		n	6	6
		4 / week		
		mean	8.00	.49
		sd	1.31	.08
		n	6	6
	HighConc	2 / week		
		mean	7.64	.47
		sd	.80	.05
		n	6	6
		4 / week		
		mean	8.03	.50
		sd	1.56	.10
		n	6	6
	PosCont	4 / week		
		mean	8.18	.49
		sd	1.22	.08
		n	8	8

TABLE 31  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
 (Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	VCPV	CCHORD
Post-Rec	Control	4 / week		
		mean	8.72	.55
		sd	1.47	.10
		n	16	16
	LowConc	2 / week		
		mean	7.96	.51
		sd	1.76	.11
		n	6	6
		4 / week		
		mean	8.11	.49
		sd	1.35	.13
		n	6	6
	HighConc	2 / week		
		mean	8.42	.54
		sd	1.07	.08
		n	6	6
		4 / week		
		mean	8.57	.54
		sd	.56	.05
		n	5	5
	PosCont	4 / week		
		mean	7.81	.49
		sd	1.31	.06
		n	7	7



TABLE 32  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Frequency and Duration*  
(averaging over exposure period and sex)

concentration	frequency (exp/week)	duration (hr/day)	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1		(1,2)
100 mg/m <sup>3</sup>	2	4		
100 mg/m <sup>3</sup>	4			
100 mg/m <sup>3</sup>	4	4		(2)
200 mg/m <sup>3</sup>	2	1		
200 mg/m <sup>3</sup>	2	4		
200 mg/m <sup>3</sup>	4	1		
200 mg/m <sup>3</sup>	4	4		(1,2)
positive control				(2)

*Key for significant effects*

1 = VCPV, 2 = CCHORD

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 33  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	VCPV	CCHORD
Control	4 / week	4 Hr/Day		
		mean	8.61	.54
		sd	1.44	.09
		n	32	32
LowConc	2 / week	1 Hr/Day		
		mean	7.23	.46
		sd	.95	.05
		n	6	6
	4 Hr/Day			
		mean	8.95	.57
		sd	.74	.05
		n	6	6
	4 / week	1 Hr/Day		
		mean	7.94	.50
		sd	1.26	.09
		n	6	6
	4 Hr/Day			
		mean	8.18	.48
		sd	1.39	.12
		n	6	6

TABLE 33  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	VCPV	CCHORD
HighConc	2 / week	1 Hr/Day		
			mean	.51
			sd	.10
			n	6
		4 Hr/Day		
			mean	.50
			sd	.03
			n	6
	4 / week	1 Hr/Day		
			mean	.56
			sd	.05
			n	5
		4 Hr/Day		
			mean	.49
			sd	.09
			n	6
PosCont	4 / week	4 Hr/Day		
			mean	.49
			sd	.07
			n	15

TABLE 34  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period*  
(averaging over sex, frequency and duration)

		significant increases	significant decreases
<i>Post-Exposure</i>	concentration		
	100 mg/m <sup>3</sup>		
	200 mg/m <sup>3</sup>		(1)
	positive control		
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>		(1)
	200 mg/m <sup>3</sup>		
	positive control		(1)

*Key for significant effects*

1 = FVC, 2 = FEV100, 3 = FEF50

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 35  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration Subgroups  
(Averaging over Sex, Frequency, and Duration)

PERIOD	GROUP	FVC	FEV100	FEF50	
Post-Exp	Control				
		mean	8.02	5.88	78.41
		sd	1.00	.64	9.41
		n	16	16	16
	LowConc				
		mean	7.61	5.60	75.50
		sd	.77	.51	12.56
		n	12	12	12
	HighConc				
		mean	7.45	5.41	73.27
		sd	.94	.63	11.72
		n	12	12	12
	PosCont				
		mean	7.91	5.88	75.45
		sd	1.12	.40	16.50
		n	8	8	8
Post-Rec	Control				
		mean	8.41	6.31	85.74
		sd	1.37	.67	6.00
		n	16	16	16
	LowConc				
		mean	7.78	6.06	82.06
		sd	1.22	.64	6.81
		n	12	12	12
	HighConc				
		mean	8.20	6.29	82.21
		sd	.90	.76	15.81
		n	11	11	11
	PosCont				
		mean	7.48	6.05	89.18
		sd	1.32	.49	5.21
		n	7	7	7

TABLE 36  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Duration*  
(averaging over sex and frequency)

	concentration	duration (hr/day)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	1		
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4		
	positive control			
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	1		
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4		
	positive control			

*Key for significant effects*

1 = FVC, 2 = FEV100, 3 = FEF50

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 37  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	FVC	FEV100	FEF50
Post-Exp	Control	4 Hr/Day			
		mean	8.02	5.88	78.41
		sd	1.00	.64	9.41
		n	16	16	16
	LowConc	1 Hr/Day			
		mean	7.61	5.83	80.79
		sd	1.10	.65	15.72
		n	6	6	6
		4 Hr/Day			
		mean	7.61	5.37	70.22
		sd	.32	.12	5.71
		n	6	6	6
	HighConc	1 Hr/Day			
		mean	7.36	5.35	71.25
		sd	1.20	.73	12.54
		n	6	6	6
		4 Hr/Day			
		mean	7.53	5.47	75.30
		sd	.69	.59	11.61
		n	6	6	6
	PosCont	4 Hr/Day			
		mean	7.91	5.88	75.45
		sd	1.12	.40	16.50
		n	8	8	8

TABLE 37  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
 (Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	FVC	FEV100	FEF50
Post-Rec	Control	4 Hr/Day			
		mean	8.41	6.31	85.74
		sd	1.37	.67	6.00
		n	16	16	16
	LowConc	1 Hr/Day			
		mean	7.69	6.22	85.84
		sd	1.49	.81	5.76
		n	6	6	6
		4 Hr/Day			
		mean	7.88	5.90	78.27
		sd	1.01	.44	5.86
		n	6	6	6
	HighConc	1 Hr/Day			
		mean	8.15	6.17	80.76
		sd	1.03	1.03	23.80
		n	5	5	5
		4 Hr/Day			
		mean	8.24	6.39	83.43
		sd	.87	.51	6.55
		n	6	6	6
	PosCont	4 Hr/Day			
		mean	7.48	6.05	89.18
		sd	1.32	.49	5.21
		n	7	7	7



TABLE 38  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Frequency*  
(averaging over sex and duration)

	concentration	frequency (exp/week)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	2		
	100 mg/m <sup>3</sup>	4		(1),(2)
	200 mg/m <sup>3</sup>	2		(1,2)
	200 mg/m <sup>3</sup>	4		
	positive control			
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	2		(1)
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4		
	positive control			(1)

*Key for significant effects*

1 = FVC, 2 = FEV100, 3 = FEF50

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 39  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	FVC	FEV100	FEF50
Post-Exp	Control	4 / week			
		mean	8.02	5.88	78.41
		sd	1.00	.64	9.41
		n	16	16	16
	LowConc	2 / week			
		mean	7.99	5.85	78.92
		sd	.72	.58	14.82
		n	6	6	6
		4 / week			
		mean	7.22	5.35	72.09
		sd	.66	.29	9.96
		n	6	6	6
	HighConc	2 / week			
		mean	7.11	5.14	69.89
		sd	.91	.80	15.24
		n	6	6	6
		4 / week			
		mean	7.79	5.68	76.66
		sd	.91	.27	6.51
		n	6	6	6
	PosCont	4 / week			
		mean	7.91	5.88	75.45
		sd	1.12	.40	16.50
		n	8	8	8

TABLE 39  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	FVC	FEV100	FEF50
Post-Rec	Control	4 / week			
		mean	8.41	6.31	85.74
		sd	1.37	.67	6.00
		n	16	16	16
	LowConc	2 / week			
		mean	7.54	5.93	85.53
		sd	1.32	.39	6.22
		n	6	6	6
		4 / week			
		mean	8.03	6.19	78.59
		sd	1.18	.85	5.86
		n	6	6	6
	HighConc	2 / week			
		mean	8.16	6.37	87.04
		sd	.89	.74	12.71
		n	6	6	6
		4 / week			
		mean	8.25	6.20	76.42
		sd	1.02	.85	18.61
		n	5	5	5
	PosCont	4 / week			
		mean	7.48	6.05	89.18
		sd	1.32	.49	5.21
		n	7	7	7

TABLE 40  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Frequency and Duration*  
(averaging over exposure period and sex)

concentration	frequency (exp/week)	duration (hr/day)	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1		
100 mg/m <sup>3</sup>	2	4		
100 mg/m <sup>3</sup>	4	1		
100 mg/m <sup>3</sup>	4	4		
200 mg/m <sup>3</sup>	2	1		
200 mg/m <sup>3</sup>	2	4		
200 mg/m <sup>3</sup>	4	1		
200 mg/m <sup>3</sup>	4	4		
positive control				

*Key for significant effects*

1 = FVC, 2 = FEV100, 3 = FEF50

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 41  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
(Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	FVC	FEV100	FEF50
Control	4 / week	4 Hr/Day			
		mean	8.22	6.10	82.08
		sd	1.19	.68	8.61
		n	32	32	32
LowConc	2 / week	1 Hr/Day			
		mean	7.48	5.98	87.19
		sd	1.22	.44	13.25
		n	6	6	6
	4 Hr/Day				
		mean	8.05	5.80	77.26
		sd	.83	.52	7.05
		n	6	6	6
	4 / week	1 Hr/Day			
		mean	7.82	6.07	79.44
		sd	1.37	.99	9.17
		n	6	6	6
	4 Hr/Day				
		mean	7.43	5.47	71.24
		sd	.49	.21	5.87
		n	6	6	6

TABLE 41  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	FVC	FEV100	FEF50	
HighConc	2 / week	1 Hr/Day				
			mean	7.53	5.71	78.15
			sd	1.45	1.27	20.95
			n	6	6	6
		4 Hr/Day				
			mean	7.74	5.80	78.78
			sd	.40	.70	11.44
			n	6	6	6
	4 / week	1 Hr/Day				
			mean	7.94	5.73	72.48
			sd	.74	.44	15.94
			n	5	5	5
		4 Hr/Day				
			mean	8.04	6.06	79.94
			sd	1.15	.77	9.26
			n	6	6	6
PosCont	4 / week	4 Hr/Day				
			mean	7.71	5.96	81.85
			sd	1.20	.44	14.07
			n	15	15	15

TABLE 42  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period*  
(averaging over sex, frequency and duration)

		significant increases	significant decreases
<i>Post-Exposure</i>	concentration		
	100 mg/m <sup>2</sup>	(1)	
	200 mg/m <sup>3</sup>	(1)	
	positive control	1	
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>		(4),[4]
	200 mg/m <sup>3</sup>		
	positive control	1	2.(4).[4].5

*Key for significant effects*

1 = FOB, 2 = VT, 3 = PES, 4 = RL, 5 = CDYN

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 43  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration Subgroups  
(Averaging over Sex, Frequency, and Duration)

PERIOD	GROUP	FOB	VT	PES	RL	CDYN
Post-Exp	Control					
	mean	81.74	1.53	6.39	.12	.31
	sd	26.00	.39	1.63	.05	.16
	n	16	16	16	15	16
	LowConc					
	mean	94.18	1.54	6.84	.13	.26
	sd	19.87	.40	1.27	.07	.06
	n	12	12	12	11	12
	HighConc					
	mean	90.39	1.47	6.48	.11	.26
	sd	20.78	.23	1.30	.04	.06
	n	12	12	12	11	12
	PosCont					
	mean	120.53	1.38	7.14	.11	.26
	sd	32.48	.24	1.41	.05	.12
	n	8	8	8	5	8
Post-Rec	Control					
	mean	89.59	1.48	7.94	.19	.24
	sd	21.86	.31	1.02	.08	.09
	n	16	16	16	16	16
	LowConc					
	mean	89.11	1.34	7.34	.13	.22
	sd	21.73	.20	1.09	.05	.05
	n	12	12	12	11	12
	HighConc					
	mean	94.13	1.43	7.19	.14	.23
	sd	20.89	.22	.76	.05	.05
	n	11	11	11	10	11
	PosCont					
	mean	123.59	1.10	8.89	.12	.14
	sd	27.76	.30	1.63	.05	.03
	n	7	7	7	7	7



TABLE 44  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Duration*  
(averaging over sex and frequency)

	concentration	duration (hr/day)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	1		
	100 mg/m <sup>3</sup>	4	(1)	
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4	(1)	
	positive control		1	
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	1		3.4
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4		4
	positive control		1	(2).[4].(4).5

*Key for significant effects*

1 = FOB, 2 = VT, 3 = PES, 4 = RL, 5 = CDYN

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 45  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	FOB	VT	PES	RL	CDYN
Post-Exp	Control	4 Hr/Day					
		mean	81.74	1.53	6.39	.12	.31
		sd	26.00	.39	1.63	.05	.16
		n	16	16	16	15	16
	LowConc	1 Hr/Day					
		mean	88.96	1.61	6.08	.08	.29
		sd	22.29	.41	1.26	.07	.05
		n	6	6	6	5	6
	4 Hr/Day						
		mean	99.39	1.48	7.61	.17	.22
		sd	17.50	.42	.75	.03	.06
		n	6	6	6	6	6
	HighConc	1 Hr/Day					
		mean	84.79	1.40	6.96	.13	.25
		sd	18.98	.15	1.67	.04	.07
		n	6	6	6	6	6
	4 Hr/Day						
		mean	95.99	1.54	6.00	.10	.28
		sd	22.68	.29	.61	.05	.05
		n	6	6	6	5	6
	PosCont	4 Hr/Day					
		mean	120.53	1.38	7.14	.11	.26
		sd	32.48	.24	1.41	.05	.12
		n	8	8	8	5	8

TABLE 45  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
 (Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	FOB	VT	PES	RL	CDYN
Post-Rec	Control	4 Hr/Day					
		mean	89.59	1.48	7.94	.19	.24
		sd	21.86	.31	1.02	.08	.09
		n	16	16	16	16	16
	LowConc	1 Hr/Day					
		mean	93.70	1.34	6.82	.09	.23
		sd	27.46	.28	.58	.03	.07
		n	6	6	6	5	6
		4 Hr/Day					
		mean	84.53	1.34	7.86	.16	.21
		sd	15.32	.10	1.27	.04	.03
		n	6	6	6	6	6
	HighConc	1 Hr/Day					
		mean	93.56	1.43	7.05	.15	.24
		sd	25.92	.18	.96	.04	.04
		n	5	5	5	5	5
		4 Hr/Day					
		mean	94.60	1.42	7.31	.13	.23
		sd	18.30	.27	.61	.06	.06
		n	6	6	6	5	6
	PosCont	4 Hr/Day					
		mean	123.59	1.10	8.89	.12	.14
		sd	27.76	.30	1.63	.05	.03
		n	7	7	7	7	7

TABLE 46  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Frequency*  
(averaging over sex and duration)

	concentration	frequency (exp/week)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	2	(1)	
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4		
	positive control		1	
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	2		(2,4)
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4		
	positive control		1	2,(4),5

*Key for significant effects*

1 = FOB, 2 = VT, 3 = PES, 4 = RL, 5 = CDYN

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 47  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	FOB	VT	PES	RL	CDYN
Post-Exp	Control	4 / week					
		mean	81.74	1.53	6.39	.12	.31
		sd	26.00	.39	1.63	.05	.16
		n	16	16	16	15	16
	LowConc	2 / week					
		mean	96.90	1.52	6.90	.10	.24
		sd	15.48	.48	.78	.08	.08
		n	6	6	6	6	6
		4 / week					
		mean	91.45	1.57	6.79	.16	.27
		sd	24.71	.35	1.71	.05	.05
		n	6	6	6	5	6
	HighConc	2 / week					
		mean	90.98	1.53	6.76	.14	.27
		sd	24.87	.30	1.48	.04	.08
		n	6	6	6	6	6
		4 / week					
		mean	89.81	1.40	6.19	.08	.26
		sd	18.17	.14	1.16	.02	.05
		n	6	6	6	5	6
	PosCont	4 / week					
		mean	120.53	1.38	7.14	.11	.26
		sd	32.48	.24	1.41	.05	.12
		n	8	8	8	5	8

TABLE 47  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
 (Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	FOB	VT	PES	RL	CDYN
Post-Rec	Control	4 / week					
		mean	89.59	1.48	7.94	.19	.24
		sd	21.86	.31	1.02	.08	.09
		n	16	16	16	16	16
	LowConc	2 / week					
		mean	82.97	1.25	7.64	.11	.19
		sd	17.63	.17	1.22	.03	.04
		n	6	6	6	5	6
		4 / week					
		mean	95.26	1.44	7.04	.14	.25
		sd	25.25	.20	.95	.06	.05
		n	6	6	6	6	6
	HighConc	2 / week					
		mean	96.13	1.40	7.04	.15	.24
		sd	19.62	.24	.72	.03	.05
		n	6	6	6	5	6
		4 / week					
		mean	91.73	1.46	7.38	.14	.22
		sd	24.44	.23	.85	.07	.05
		n	5	5	5	5	5
	PosCont	4 / week					
		mean	123.59	1.10	8.89	.12	.14
		sd	27.76	.30	1.63	.05	.03
		n	7	7	7	7	7

TABLE 48  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Frequency and Duration*  
(averaging over exposure period and sex)

concentration	frequency (exp/week)	duration (hr/day)	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1		4
100 mg/m <sup>3</sup>	2	4		
100 mg/m <sup>3</sup>	4	1		
100 mg/m <sup>3</sup>	4	4		
200 mg/m <sup>3</sup>	2	1		
200 mg/m <sup>3</sup>	2	4		
200 mg/m <sup>3</sup>	4	1		
200 mg/m <sup>3</sup>	4	4		4
positive control			1	

*Key for significant effects*

1 = FOB, 2 = VT, 3 = PES, 4 = RL, 5 = CDYN

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 49  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	FOB	VT	PES	RL	CDYN
Control	4 / week	4 Hr/Day					
			mean	85.66	1.51	7.16	.15
			sd	23.96	.35	1.55	.07
			n	32	32	32	31
LowConc	2 / week	1 Hr/Day					
			mean	90.63	1.52	6.98	.06
			sd	24.22	.48	.65	.04
			n	6	6	6	5
		4 Hr/Day					
			mean	89.23	1.25	7.56	.14
			sd	8.89	.17	1.34	.04
			n	6	6	6	6
	4 / week	1 Hr/Day					
			mean	92.03	1.43	5.92	.11
			sd	26.00	.24	1.07	.05
			n	6	6	6	5
		4 Hr/Day					
			mean	94.68	1.58	7.91	.18
			sd	24.01	.32	.58	.02
			n	6	6	6	6



TABLE 49  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	FOB	VT	PES	RL	CDYN	
HighConc	2 / week	1 Hr/Day						
			mean	90.22	1.40	6.99	.15	.24
			sd	24.72	.16	1.44	.03	.06
			n	6	6	6	6	6
		4 Hr/Day						
			mean	96.89	1.54	6.81	.14	.27
			sd	19.54	.34	.80	.04	.07
			n	6	6	6	5	6
	4 / week	4 Hr/Day						
			mean	87.05	1.44	7.01	.13	.24
			sd	20.10	.17	1.35	.05	.07
			n	5	5	5	5	5
		4 Hr/Day						
			mean	93.71	1.42	6.50	.09	.25
			sd	21.51	.20	1.04	.06	.05
			n	6	6	6	5	6
PosCont	4 / week	4 Hr/Day						
			mean	121.96	1.25	7.95	.12	.21
			sd	29.33	.30	1.72	.05	.11
			n	15	15	15	12	15

TABLE 50  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period*  
(averaging over sex, frequency and duration)

		significant increases	significant decreases
<i>Post-Exposure</i>	concentration		
	100 mg/m <sup>3</sup>		(1,3)
	200 mg/m <sup>3</sup>		(1,3)
	positive control		(1,2,3)
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>		(2,3)
	200 mg/m <sup>3</sup>		
	positive control		2

*Key for significant effects*

1 = TLC, 2 = DLCO, 3 = VC

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 51  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration Subgroups  
(Averaging over Sex, Frequency, and Duration)

PERIOD	GROUP	TLC	DLCO	VC
Post-Exp	Control			
	mean	9.46	.14	8.10
	sd	1.06	.03	.93
	n	16	16	15
	LowConc			
	mean	8.87	.13	7.65
	sd	.87	.03	.67
	n	11	11	11
	HighConc			
	mean	8.39	.12	7.34
	sd	.93	.03	.71
	n	11	11	11
	PosCont			
	mean	8.55	.10	7.59
	sd	1.06	.02	.96
	n	8	8	8
Post-Rec	Control			
	mean	9.82	.14	8.56
	sd	1.49	.04	1.29
	n	16	16	16
	LowConc			
	mean	9.16	.12	8.03
	sd	1.26	.03	1.05
	n	12	12	12
	HighConc			
	mean	9.69	.13	8.41
	sd	1.07	.03	.83
	n	11	11	11
	PosCont			
	mean	9.14	.09	8.24
	sd	1.29	.02	1.15
	n	7	7	7

TABLE 52  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Duration*  
(averaging over sex and frequency)

	concentration	duration (hr/day)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	1		(1,3)
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	1		(1,3)
	200 mg/m <sup>3</sup>	4		(1,3)
	positive control			(1,2,3)
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	1		(2)
	100 mg/m <sup>3</sup>	4		(2)
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4		(2)
	positive control			2

*Key for significant effects*

1 = TLC, 2 = DLCO, 3 = VC

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , bold text:  $p < .01$

TABLE 53  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	TLC	DLCO	VC
Post-Exp	Control	4 Hr/Day			
		mean	9.46	.14	8.10
		sd	1.06	.03	.93
		n	16	16	15
	LowConc	1 Hr/Day			
		mean	8.67	.13	7.52
		sd	1.05	.03	.78
		n	5	5	5
	LowConc	4 Hr/Day			
		mean	9.03	.13	7.77
		sd	.74	.03	.61
		n	6	6	6
	HighConc	1 Hr/Day			
		mean	8.38	.12	7.32
		sd	1.05	.02	.76
		n	5	5	5
	HighConc	4 Hr/Day			
		mean	8.40	.13	7.35
		sd	.93	.04	.74
		n	6	6	6
	PosCont	4 Hr/Day			
		mean	8.55	.10	7.59
		sd	1.06	.02	.96
		n	8	8	8

TABLE 53  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
 (Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	TLC	DLCO	VC
Post-Rec	Control	4 Hr/Day			
		mean	9.82	.14	8.56
		sd	1.49	.04	1.29
		n	16	16	16
	LowConc	1 Hr/Day			
		mean	9.02	.12	7.83
		sd	1.41	.03	1.16
		n	6	6	6
		4 Hr/Day			
		mean	9.32	.12	8.22
		sd	1.22	.02	.98
		n	6	6	6
	HighConc	1 Hr/Day			
		mean	9.83	.14	8.44
		sd	1.31	.04	.97
		n	5	5	5
		4 Hr/Day			
		mean	9.57	.12	8.38
		sd	.92	.03	.80
		n	6	6	6
	PosCont	4 Hr/Day			
		mean	9.14	.09	8.24
		sd	1.29	.02	1.15
		n	7	7	7

TABLE 54  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Frequency*  
(averaging over sex and duration)

	concentration	frequency (exp/week)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	2		
	100 mg/m <sup>3</sup>	4		(1,3)
	200 mg/m <sup>3</sup>	2		(1,3)
	200 mg/m <sup>3</sup>	4		(1),(2),(3)
	positive control			(1,2,3)
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	2		(1),(2)
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4		(1)
	positive control			2

*Key for significant effects*

1 = TLC, 2 = DLCO, 3 = VC

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 55  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	TLC	DLCO	VC
Post-Exp	Control	4 / week			
		mean	9.46	.14	8.10
		sd	1.06	.03	.93
		n	16	16	15
	LowConc	2 / week			
		mean	8.96	.13	7.68
		sd	.81	.04	.64
		n	5	5	5
		4 / week			
		mean	8.79	.13	7.63
		sd	.98	.03	.75
		n	6	6	6
	HighConc	2 / week			
		mean	8.44	.13	7.38
		sd	.96	.03	.78
		n	6	6	6
		4 / week			
		mean	8.33	.12	7.28
		sd	1.01	.03	.70
		n	5	5	5
	PosCont	4 / week			
		mean	8.55	.10	7.59
		sd	1.06	.02	.96
		n	8	8	8



TABLE 55  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	TLC	DLCO	VC
Post-Rec	Control	4 / week			
		mean	9.82	.14	8.56
		sd	1.49	.04	1.29
		n	16	16	16
	LowConc	2 / week			
		mean	9.03	.12	7.92
		sd	1.46	.03	1.29
		n	6	6	6
		4 / week			
		mean	9.31	.12	8.13
		sd	1.16	.03	.85
		n	6	6	6
	HighConc	2 / week			
		mean	9.69	.13	8.33
		sd	1.23	.04	.90
		n	6	6	6
		4 / week			
		mean	9.69	.13	8.50
		sd	.98	.03	.84
		n	5	5	5
	PosCont	4 / week			
		mean	9.14	.09	8.24
		sd	1.29	.02	1.15
		n	7	7	7

TABLE 56  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Frequency and Duration*  
(averaging over exposure period and sex)

concentration	frequency (exp/week)	duration (hr/day)	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1		(1,3)
100 mg/m <sup>3</sup>	2	4		
100 mg/m <sup>3</sup>	4	1		(1,3)
100 mg/m <sup>3</sup>	4	4		(1,3)
200 mg/m <sup>3</sup>	2	1		
200 mg/m <sup>3</sup>	2	4		(1,3)
200 mg/m <sup>3</sup>	4	1		(3)
200 mg/m <sup>3</sup>	4	4		(1),(2),(3)
positive control				(1),2,(3)

*Key for significant effects*

1 = TLC, 2 = DLCO, 3 = VC

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 57  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	TLC	DLCO	VC
Control	4 / week	4 Hr/Day			
		mean	9.64	.14	8.34
		sd	1.28	.03	1.14
		n	32	32	31
LowConc	2 / week	1 Hr/Day			
		mean	8.55	.12	7.40
		sd	1.10	.04	.86
		n	5	5	5
		4 Hr/Day			
		mean	9.36	.12	8.15
		sd	1.15	.02	1.06
		n	6	6	6
	4 / week	1 Hr/Day			
		mean	9.11	.13	7.93
		sd	1.34	.02	1.07
		n	6	6	6
		4 Hr/Day			
		mean	8.98	.13	7.83
		sd	.81	.04	.53
		n	6	6	6

TABLE 57  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENCY	DURATION	TLC	DLCO	VC	
HighConc	2 / week	1 Hr/Day				
			mean	9.12	.13	7.88
			sd	1.78	.03	1.33
			n	6	6	6
		4 Hr/Day				
			mean	9.01	.13	7.83
			sd	.43	.04	.44
			n	6	6	6
	4 / week	1 Hr/Day				
			mean	9.09	.13	7.88
			sd	.49	.03	.32
			n	4	4	4
		4 Hr/Day				
			mean	8.96	.12	7.90
			sd	1.53	.03	1.28
			n	6	6	6
PosCont	4 / week	4 Hr/Day				
			mean	8.83	.10	7.89
			sd	1.17	.02	1.07
			n	15	15	15

TABLE 58  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period*  
(averaging over sex, frequency and duration)

		significant increases	significant decreases
<i>Post-Exposure</i>	concentration		
	100 mg/m <sup>3</sup>	(10),	(3),(4),(12),
	200 mg/m <sup>3</sup>	(10),	(3,4),[7],[11],[12],15,
	positive control	(10),	(11),12,15,
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>		(3,4),
	200 mg/m <sup>3</sup>		
	positive control		(3,4),11,(12),15,

*Key for significant effects*

1 = CPK, 2 = FEV50, 3 = FEV200, 4 = FEV400, 5 = PEXF, 6 = VPEXF,

7 = MMEXF, 8 = FEF25, 9 = FEF75, 10 = VE, 11 = TI, 12 = TE,

13 = VEMAX, 14 = VIMAX, 15 = RV

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 59  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration Subgroups  
(Averaging over Sex, Frequency, and Duration)

PERIOD	GROUP	CPK	FEV50	FEV200	FEV400	PEXF	VPEXF	MMEXF
Post-Exp	Control							
	mean	.87	2.66	7.59	7.95	88.24	2.71	68.47
	sd	.14	.50	.86	.94	14.19	.49	5.46
	n	14	16	16	16	16	16	16
	LowConc							
	mean	.83	2.52	7.13	7.49	85.23	2.56	64.81
	sd	.08	.55	.73	.76	18.37	.68	5.64
	n	9	12	12	12	12	12	12
	HighConc							
	mean	.84	2.37	7.03	7.36	79.59	2.63	63.71
	sd	.16	.51	.81	.89	15.36	.74	7.54
	n	12	12	12	12	12	12	12
PosCont								
mean	.81	2.62	7.37	7.76	85.16	2.46	70.48	
sd	.15	.38	.88	.99	10.86	.22	6.38	
n	7	8	8	8	8	8	8	
Post-Rec	Control							
	mean	.90	2.83	7.98	8.30	93.74	2.73	75.62
	sd	.16	.46	1.18	1.30	18.28	.52	7.05
	n	16	16	16	16	16	16	16
	LowConc							
	mean	.84	2.89	7.41	7.70	94.45	2.29	73.76
	sd	.17	.52	1.06	1.18	18.10	.47	6.92
	n	12	12	12	12	12	12	12
	HighConc							
	mean	.88	2.92	7.79	8.10	94.99	2.36	74.01
	sd	.09	.51	.88	.88	15.78	.55	12.43
	n	11	11	11	11	11	11	11
PosCont								
mean	.74	2.88	7.21	7.43	92.45	2.54	77.89	
sd	.11	.19	1.11	1.22	5.33	.59	5.51	
n	5	7	7	7	7	7	7	

TABLE 59 (continued)  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration Subgroups  
(Averaging over Sex, Frequency, and Duration)

PERIOD	GROUP	FEF25	FEF75	VE	TI	TE	TEMPX	VIMAX
Post-Exp	Control	mean	42.12	85.68	125.73	.30	.50	11.24
		sd	4.63	13.98	46.35	.06	.20	2.04
		n	16	16	16	16	16	16
	LowConc	mean	40.39	83.01	150.93	.28	.37	10.51
		sd	6.52	18.17	58.56	.02	.11	1.99
		n	12	12	12	12	12	12
	HighConc	mean	41.98	77.53	137.39	.27	.41	10.06
		sd	10.50	15.33	46.27	.03	.13	1.65
		n	12	12	12	12	12	12
	PosCont	mean	40.16	77.55	170.53	.25	.29	11.58
		sd	12.47	18.22	61.36	.05	.14	3.07
		n	8	8	8	8	8	8
Post-Rec	Control	mean	46.74	91.80	137.55	.29	.42	11.92
		sd	5.67	17.62	55.06	.04	.15	2.57
		n	16	16	16	16	16	16
	LowConc	mean	47.49	91.78	123.49	.28	.43	11.39
		sd	5.69	16.08	46.68	.03	.16	2.43
		n	12	12	12	12	12	12
	HighConc	mean	47.45	93.61	136.78	.27	.40	11.65
		sd	10.44	15.90	41.12	.03	.15	1.92
		n	11	11	11	11	11	11
	PosCont	mean	51.09	90.82	137.20	.24	.27	11.09
		sd	8.53	6.07	45.05	.05	.08	2.35
		n	7	7	7	7	7	7

TABLE 60  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Duration*  
(averaging over sex and frequency)

	concentration	duration (hr/day)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	1		(3,12).
	100 mg/m <sup>3</sup>	4	(10),	(3,7).(12).
	200 mg/m <sup>3</sup>	1		(3,4).
	200 mg/m <sup>3</sup>	4	(10),	(3,12,15).
	positive control		(10),	(12),15.
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	1		
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	1		
	200 mg/m <sup>3</sup>	4		
	positive control			11,(12),15.

*Key for significant effects*

1 = CPK, 2 = FEV50, 3 = FEV200, 4 = FEV400, 5 = PEXF, 6 = VPEXF.

7 = MMEXF, 8 = FEF25, 9 = FEF75, 10 = VE, 11 = TI, 12 = TE,

13 = VEMAX, 14 = VIMAX, 15 = RV

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$



TABLE 61  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	CPK	FEV50	FEV200	FEV400	PEXF	VPEXF	MMEXF		
Post-Exp	Control	4 Hr/Day									
			mean	.87	2.66	7.59	7.95	88.24	2.71	68.47	
			sd	.14	.50	.86	.94	14.19	.49	5.46	
		n	14	16	16	16	16	16	16		
		LowConc	1 Hr/Day								
				mean	.79	2.85	7.22	7.53	96.49	2.52	67.89
				sd	.07	.60	1.01	1.08	19.54	.78	6.43
			n	5	6	6	6	6	6	6	
			4 Hr/Day								
	mean			.89	2.19	7.05	7.46	73.98	2.60	61.73	
	sd			.07	.23	.34	.33	7.55	.65	2.42	
	n			4	6	6	6	6	6	6	
	HighConc			1 Hr/Day							
		mean			.84	2.22	6.99	7.30	74.22	2.83	63.01
		sd	.12		.37	1.02	1.15	13.20	.91	8.07	
		n	6	6	6	6	6	6	6		
		4 Hr/Day									
			mean	.85	2.52	7.07	7.42	84.95	2.42	64.40	
			sd	.20	.61	.61	.63	16.60	.52	7.66	
			n	6	6	6	6	6	6	6	
			PosCont	4 Hr/Day							
	mean				.81	2.62	7.37	7.76	85.16	2.46	70.48
	sd	.15			.38	.88	.99	10.86	.22	6.38	
	n	7		8	8	8	8	8	8		

TABLE 61  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Summary  
(Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	CPK	FEV50	FEV200	FEV400	PEXF	VPEXF	MMEXF
Post-Rec	Control	4 Hr/Day							
		mean	.90	2.83	7.98	8.30	93.74	2.73	75.62
		sd	.16	.46	1.18	1.30	18.28	.52	7.05
		n	16	16	16	16	16	16	16
	LowConc	1 Hr/Day							
		mean	.81	3.11	7.34	7.61	103.22	2.19	79.09
		sd	.19	.63	1.30	1.44	22.15	.51	4.86
		n	6	6	6	6	6	6	6
		4 Hr/Day							
		mean	.88	2.67	7.47	7.79	85.67	2.39	68.43
		sd	.16	.27	.88	.99	6.71	.44	3.67
		n	6	6	6	6	6	6	6
	HighConc	1 Hr/Day							
		mean	.91	2.93	7.72	8.06	93.60	2.18	71.26
		sd	.09	.39	1.14	1.08	12.41	.48	18.54
		n	5	5	5	5	5	5	5
		4 Hr/Day							
		mean	.86	2.91	7.86	8.13	96.14	2.51	76.30
		sd	.09	.63	.69	.79	19.26	.60	4.50
		n	6	6	6	6	6	6	6
	PosCont	4 Hr/Day							
		mean	.74	2.88	7.21	7.43	92.45	2.54	77.89
		sd	.11	.19	1.11	1.22	5.33	.59	5.51
		n	5	7	7	7	7	7	7

TABLE 61 (continued)  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Duration Subgroups  
(Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	FEF25	FEF75	VE	TI	TE	VEMAX	VIMAX	RV	
Post-Exp	Control	4 Hr/Day									
		mean	42.12	85.68	125.73	.30	.50	-8.91	11.24	1.28	
		sd	4.63	13.98	46.35	.06	.20	1.79	2.04	.24	
		n	16	16	16	16	16	16	16	16	
	LowConc	1 Hr/Day									
		mean	41.87	93.96	150.80	.29	.39	-8.83	9.93	1.15	
		sd	4.68	19.84	61.96	.02	.14	2.33	2.20	.28	
		n	6	6	6	6	6	6	6	5	
		4 Hr/Day									
		mean	38.91	72.06	151.06	.27	.35	-9.57	11.08	1.27	
		sd	8.15	6.73	60.88	.02	.09	2.46	1.75	.17	
		n	6	6	6	6	6	6	6	6	
HighConc	1 Hr/Day										
	mean	43.45	72.20	122.77	.28	.42	-8.85	10.87	1.06		
	sd	10.99	12.17	32.94	.02	.12	1.57	1.65	.33		
	n	6	6	6	6	6	6	6	5		
	4 Hr/Day										
	mean	40.51	82.86	152.01	.27	.39	-9.73	9.25	1.05		
	sd	10.80	17.35	55.79	.04	.14	2.54	1.32	.27		
	n	6	6	6	6	6	6	6	6		
PosCont	4 Hr/Day										
	mean	40.16	77.55	170.53	.25	.29	-9.98	11.58	.97		
	sd	12.47	18.22	61.36	.05	.14	2.19	3.07	.14		
	n	8	8	8	8	8	8	8	8		

TABLE 61 (continued)  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Period by Concentration by Duration subgroups  
 (Averaging over Sex and Frequency)

PERIOD	GROUP	DURATION	FEF25	FEF75	VE	TI	TE	VEMAX	VIMAX	RV
Post-Rec	Control	4 Hr/Day								
		mean	46.74	91.80	137.55	.29	.42	-9.17	11.92	1.26
		sd	5.67	17.62	55.06	.04	.15	2.16	2.57	.22
		n	16	16	16	16	16	16	16	
	LowConc	1 Hr/Day								
		mean	50.84	99.62	132.21	.28	.41	-9.05	12.16	1.18
		sd	6.31	19.14	63.75	.04	.19	2.14	3.13	.27
		n	6	6	6	6	6	6	6	
		4 Hr/Day								
		mean	44.15	83.94	114.78	.29	.45	-8.53	10.63	1.10
		sd	2.15	7.42	23.40	.02	.15	.64	1.35	.27
		n	6	6	6	6	6	6	6	
	HighConc	1 Hr/Day								
		mean	43.03	92.46	137.68	.29	.40	-8.54	11.77	1.39
		sd	13.10	11.86	47.31	.03	.21	1.22	2.41	.36
		n	5	5	5	5	5	5	5	
		4 Hr/Day								
		mean	51.14	94.56	136.03	.26	.39	-9.46	11.55	1.19
		sd	6.68	19.76	39.87	.03	.11	1.32	1.64	.16
		n	6	6	6	6	6	6	6	
	PosCont	4 Hr/Day								
		mean	51.09	90.82	137.20	.24	.27	-8.21	11.09	.90
		sd	8.53	6.07	45.05	.05	.08	2.47	2.35	.27
		n	7	7	7	7	7	7	7	

TABLE 62  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Period and Frequency*  
(averaging over sex and duration)

	concentration	frequency (exp/week)	significant increases	significant decreases
<i>Post-Exposure</i>	100 mg/m <sup>3</sup>	2		(12),
	100 mg/m <sup>3</sup>	4	(10),	(3,4),(12),
	200 mg/m <sup>3</sup>	2	(10),	(3,4),(12,15),
	200 mg/m <sup>3</sup>	4		(12),
	positive control		(10),	(12),15,
<i>Post-Recovery</i>	100 mg/m <sup>3</sup>	2		(3,4),
	100 mg/m <sup>3</sup>	4		
	200 mg/m <sup>3</sup>	2		
	200 mg/m <sup>3</sup>	4		(3,4),
	positive control			(3),(4,11,12),15,

*Key for significant effects*

1 = CPK, 2 = FEV50, 3 = FEV200, 4 = FEV400, 5 = PEXF, 6 = VPEXF,  
7 = MMEXF, 8 = FEF25, 9 = FEF75, 10 = VE, 11 = TI, 12 = TE,  
13 = VEMAX, 14 = VIMAX, 15 = RV

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment  
(bracketed text): significant difference *only* with Body Weight covariate adjustment  
[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 63  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	CPK	FEV50	FEV200	FEV400	PEXF	VPEXF	MMEXF
Post-Exp	Control	4 / week							
		mean	.87	2.66	7.59	7.95	88.24	2.71	68.47
		sd	.14	.50	.86	.94	14.19	.49	5.46
		n	14	16	16	16	16	16	16
	LowConc	2 / week							
		mean	.85	2.73	7.44	7.84	94.07	2.93	65.83
		sd	.05	.66	.81	.78	21.56	.80	5.85
		n	5	6	6	6	6	6	6
		4 / week							
		mean	.81	2.30	6.83	7.15	76.40	2.19	63.78
		sd	.11	.35	.53	.62	9.52	.23	5.77
		n	4	6	6	6	6	6	6
	HighConc	2 / week							
		mean	.87	2.12	6.82	7.07	71.91	2.55	63.15
		sd	.16	.47	.85	.89	14.82	.63	9.40
		n	6	6	6	6	6	6	6
		4 / week							
		mean	.81	2.61	7.24	7.65	87.27	2.71	64.26
		sd	.17	.45	.78	.86	12.55	.89	5.99
		n	6	6	6	6	6	6	6
	PosCont	4 / week							
		mean	.81	2.62	7.37	7.76	85.16	2.46	70.48
		sd	.15	.38	.88	.99	10.86	.22	6.38
		n	7	8	8	8	8	8	8

TABLE 63  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	CPK	FEV50	FEV200	FEV400	PEXF	VPEXF	MMEXF
Post-Rec	Control	4 / week							
		mean	.90	2.83	7.98	8.30	93.74	2.73	75.62
		sd	.16	.46	1.18	1.30	18.28	.52	7.05
		n	16	16	16	16	16	16	16
	LowConc	2 / week							
		mean	.82	2.75	7.18	7.47	88.24	2.46	75.68
		sd	.20	.15	1.13	1.28	5.08	.42	5.30
		n	6	6	6	6	6	6	6
		4 / week							
		mean	.87	3.02	7.63	7.93	100.65	2.12	71.84
		sd	.14	.72	1.03	1.13	24.54	.48	8.27
		n	6	6	6	6	6	6	6
	HighConc	2 / week							
		mean	.87	2.79	7.86	8.11	90.78	2.59	77.72
		sd	.10	.44	.86	.88	12.69	.44	11.41
		n	6	6	6	6	6	6	6
		4 / week							
		mean	.89	3.08	7.72	8.08	100.04	2.08	69.55
		sd	.07	.60	.99	.99	19.04	.57	13.34
		n	5	5	5	5	5	5	5
PosCont	4 / week								
		mean	.74	2.88	7.21	7.43	92.45	2.54	77.89
		sd	.11	.19	1.11	1.22	5.33	.59	5.51
		n	5	7	7	7	7	7	7

TABLE 63 (continued)  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	FEF25	FEF75	VE	TI	TE	VEMAX	VIMAX	RV
Post-Exp	Control	4 / week								
		mean	42.12	85.68	125.73	.30	.50	-8.91	11.24	1.28
		sd	4.63	13.98	46.35	.06	.20	1.79	2.04	.24
		n	16	16	16	16	16	16	16	
	LowConc	2 / week								
		mean	39.55	91.18	150.12	.28	.35	-9.19	10.76	1.28
		sd	5.54	21.83	61.00	.02	.09	2.00	1.63	.20
		n	6	6	6	6	6	6	6	5
		4 / week								
		mean	41.23	74.84	151.75	.28	.39	-9.20	10.26	1.16
		sd	7.83	9.48	61.83	.03	.13	2.80	2.43	.24
		n	6	6	6	6	6	6	6	6
	HighConc	2 / week								
		mean	44.95	69.66	146.86	.26	.40	-9.62	9.61	1.06
		sd	5.79	13.89	57.55	.03	.13	2.64	1.40	.27
		n	6	6	6	6	6	6	6	6
		4 / week								
		mean	39.01	85.40	127.92	.29	.41	-8.96	10.52	1.05
		sd	13.71	13.26	34.39	.03	.13	1.46	1.88	.34
		n	6	6	6	6	6	6	6	5
	PosCont	4 / week								
		mean	40.16	77.55	170.53	.25	.29	-9.98	11.58	.97
		sd	12.47	18.22	61.36	.05	.14	2.19	3.07	.14
		n	8	8	8	8	8	8	8	8



TABLE 63 (continued)  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Period by Concentration by Frequency Subgroups  
(Averaging over Sex and Duration)

PERIOD	GROUP	FREQUENCY	FEF25	FEF75	VE	TI	TE	VEMAX	VIMAX	RV
Post-Rec	Control	4 / week								
		mean	46.74	91.80	137.55	.29	.42	-9.17	11.92	1.26
		sd	5.67	17.62	55.06	.04	.15	2.16	2.57	.22
		n	16	16	16	16	16	16	16	16
	LowConc	2 / week								
		mean	47.31	86.81	104.56	.29	.47	-8.13	10.62	1.11
		sd	3.37	4.83	26.94	.03	.15	.89	1.31	.18
		n	6	6	6	6	6	6	6	6
		4 / week								
		mean	47.67	96.74	142.42	.28	.39	-9.44	12.16	1.17
		sd	7.74	22.06	56.64	.03	.19	1.82	3.14	.34
		n	6	6	6	6	6	6	6	6
	HighConc	2 / week								
		mean	51.06	89.23	136.78	.28	.37	-8.61	11.32	1.36
		sd	10.97	12.56	40.76	.04	.13	.95	1.86	.33
		n	6	6	6	6	6	6	6	6
		4 / week								
		mean	43.13	98.86	136.78	.27	.43	-9.56	12.04	1.19
		sd	8.90	19.27	46.38	.02	.19	1.58	2.13	.18
		n	5	5	5	5	5	5	5	5
	PosCont	4 / week								
		mean	51.09	90.82	137.20	.24	.27	-8.21	11.09	.90
		sd	8.53	6.07	45.05	.05	.08	2.47	2.35	.27
		n	7	7	7	7	7	7	7	7

TABLE 64  
Significant Differences for Exposed Animals as Compared to Filtered Air Controls  
*Broken Down by Frequency and Duration*  
(averaging over exposure period and sex)

concentration	frequency (exp/week)	duration (hr/day)	significant increases	significant decreases
100 mg/m <sup>3</sup>	2	1		(3,4).
100 mg/m <sup>3</sup>	2	4		
100 mg/m <sup>3</sup>	4	1		<b>6.</b>
100 mg/m <sup>3</sup>	4	4	(10),	(3,4).5,6,(7),
200 mg/m <sup>3</sup>	2	1		(3,4),[6],
200 mg/m <sup>3</sup>	2	4		[11],(11),
200 mg/m <sup>3</sup>	4	1		(3).
200 mg/m <sup>3</sup>	4	4		(3), <b>6</b> ,(15),
positive control			(10),	(3,4).11,12,(15),

*Key for significant effects*

1 = CPK, 2 = FEV50, 3 = FEV200, 4 = FEV400, 5 = PEXF, 6 = VPENF,

7 = MMEXF, 8 = FEF25, 9 = FEF75, 10 = VE, 11 = TI, 12 = TE,

13 = VEMAX, 14 = VIMAX, 15 = RV

unbracketed text: significant difference both with *and* without Body Weight covariate adjustment

(bracketed text): significant difference *only* with Body Weight covariate adjustment

[bracketed text]: significant difference *only* without Body Weight covariate adjustment

normal text:  $p < .05$ , **bold text**:  $p < .01$

TABLE 65  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
(Averaging over Exposure, Period, and Sex)

GROUP	FREQUENC	DURATION	CPK	FEV50	FEV200	FEV400	PEXF	VPEXF	MMEXF
Control	4 / week	4 Hr/Day							
		mean	.89	2.74	7.78	8.12	90.99	2.72	72.05
		sd	.15	.48	1.04	1.13	16.34	.50	7.19
		n	30	32	32	32	32	32	32
LowConc	2 / week	1 Hr/Day							
		mean	.72	3.01	7.14	7.41	100.46	2.58	75.26
		sd	.11	.43	1.09	1.20	15.84	.76	6.14
		n	5	6	6	6	6	6	6
	4 Hr/Day								
		mean	.93	2.47	7.47	7.91	81.85	2.81	66.25
		sd	.10	.31	.85	.87	7.25	.59	5.83
		n	6	6	6	6	6	6	6
	4 / week	1 Hr/Day							
		mean	.87	2.94	7.43	7.73	99.25	2.13	71.72
		sd	.13	.78	1.22	1.31	25.45	.48	9.75
		n	6	6	6	6	6	6	6
	4 Hr/Day								
		mean	.81	2.38	7.04	7.34	77.80	2.18	63.91
		sd	.14	.39	.40	.47	11.03	.24	3.00
		n	4	6	6	6	6	6	6

TABLE 65  
L06234 Pulmonary Function Analysis of All Animals  
Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
(Averaging over Exposure, Period, and Sex)

GROUP	FREQUENC	DURATION	CPK	FEV50	FEV200	FEV400	PEXF	VPEXF	MMEXF
HighConc	2 / week	1 Hr/Day							
			mean	.85	2.52	7.22	7.49	81.67	2.29
			sd	.13	.67	1.41	1.44	21.39	.56
			n	6	6	6	6	6	6
		4 Hr/Day							
			mean	.90	2.39	7.46	7.69	81.01	2.85
			sd	.13	.47	.31	.37	11.68	.30
			n	6	6	6	6	6	6
	4 / week	1 Hr/Day							
			mean	.89	2.57	7.44	7.83	84.66	2.83
			sd	.09	.33	.69	.74	7.05	.98
			n	5	5	5	5	5	5
		4 Hr/Day							
			mean	.81	3.03	7.48	7.86	100.09	2.09
			sd	.16	.64	1.06	1.09	19.15	.44
			n	6	6	6	6	6	6
PosCont	4 / week	4 Hr/Day							
			mean	.78	2.74	7.29	7.60	88.57	2.50
			sd	.13	.32	.96	1.08	9.23	.42
			n	12	15	15	15	15	15

TABLE 65 (continued)  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENC	DURATION	FEF25	FEF75	VE	TI	TE	VEMAX	VIMAX	RV
Control	4 / week	4 Hr/Day								
		mean	44.43	88.74	131.64	.30	.46	-9.04	11.58	1.27
		sd	5.61	15.95	50.42	.07	.18	1.95	2.31	.23
		n	32	32	32	32	32	32	32	32
LowConc	2 / week	1 Hr/Day								
		mean	46.49	98.47	143.28	.29	.41	-9.14	10.70	1.15
		sd	4.60	15.54	69.24	.02	.19	1.98	1.54	.25
		n	6	6	6	6	6	6	6	5
	4 Hr/Day									
		mean	40.38	79.53	111.40	.28	.41	-8.18	10.68	1.22
		sd	5.89	7.40	16.94	.02	.05	1.00	1.42	.17
		n	6	6	6	6	6	6	6	6
	4 / week	1 Hr/Day								
		mean	46.22	95.11	139.73	.28	.38	-8.73	11.39	1.18
		sd	9.42	23.05	57.53	.04	.14	2.45	3.86	.29
		n	6	6	6	6	6	6	6	6
	4 Hr/Day									
		mean	42.68	76.47	154.44	.28	.40	-9.91	11.03	1.15
		sd	7.05	11.16	60.37	.03	.18	2.08	1.71	.30
		n	6	6	6	6	6	6	6	6

TABLE 65 (continued)  
 L06234 Pulmonary Function Analysis of All Animals  
 Means, SDs, and Ns for Concentration by Frequency by Duration Subgroups  
 (Averaging over Exposure, Period, and Sex)

GROUP	FREQUENC	DURATION	FEF25	FEF75	VE	TI	TE	VEMAX	VIMAX	RV	
HighConc	2 / week	1 Hr/Day									
			mean	44.72	80.00	131.82	.28	.38	-8.14	10.95	1.23
			sd	9.73	20.90	44.91	.03	.15	1.24	2.24	.46
			n	6	6	6	6	6	6	6	
		4 Hr/Day									
			mean	51.28	78.89	151.82	.25	.39	-10.09	9.98	1.18
			sd	7.47	11.96	52.69	.04	.11	2.16	1.27	.15
			n	6	6	6	6	6	6	6	
	4 / week	1 Hr/Day									
			mean	41.49	83.10	126.82	.28	.44	-9.39	11.68	1.21
			sd	14.05	7.31	34.74	.02	.18	1.28	1.76	.25
			n	5	5	5	5	5	5	4	
		4 Hr/Day									
			mean	40.37	98.54	136.21	.28	.40	-9.10	10.82	1.06
			sd	10.10	19.98	43.96	.03	.14	1.73	2.35	.28
			n	6	6	6	6	6	6	6	
PosCont	4 / week	4 Hr/Day									
			mean	45.26	83.74	154.97	.24	.28	-9.15	11.35	.93
			sd	11.87	15.12	55.22	.05	.11	2.42	2.67	.20
			n	15	15	15	15	15	15	15	

## ATTACHMENT

### LIST OF ABBREVIATIONS

A1	- graphite test article
ALB	- albumin (grams/deciliter serum)
ALP	- alkaline phosphatase (international units/liter serum)
ALT	- alanine aminotransferase (international units/liter serum)
AM	- alveolar macrophage
BASO	- basophils (percent leukocytes counted)
BUN	- urea nitrogen (milligrams nitrogen/deciliter serum)
BWT	- body weight (grams)
°C	- degrees centigrade
C0	- filtered-air control
C1	- graphite @ 100 mg/m <sup>3</sup>
C2	- graphite @ 200 mg/m <sup>3</sup>
CP	- crystobalite @ 200 mg/m <sup>3</sup>
CA	- calcium (milligrams/deciliter serum)
Cchord	- compliance (tangent) at 0 to 10 cm H <sub>2</sub> O (ml/cm H <sub>2</sub> O)
Cdyn	- dynamic compliance (ml/cm H <sub>2</sub> O)
CHOL	- cholesterol (milligrams/deciliter serum)
cm	- centimeter
CRBC	- <sup>51</sup> Chromium-labelled chicken red blood cells
CK	- creatine kinase (international units/liter serum)
Cpk	- peak compliance (ml/cm H <sub>2</sub> O)
CREA	- Creatinine (milligrams/deciliter serum)
3DAYWK1A	- Week 1 Period 1 (BWT gain for 3 day period in grams)
4DAYWK1B	- Week 1 Period 2 (BWT gain for 4 day period in grams)
3DAYWK2A	- Week 2 Period 1 (BWT gain for 3 day period in grams)
4DAYWK2B	- Week 2 Period 2 (BWT gain for 4 day period in grams)
3DAYWK3A	- Week 3 Period 1 (BWT gain for 3 day period in grams)
4DAYWK3B	- Week 3 Period 2 (BWT gain for 4 day period in grams)
3DAYWK4A	- Week 4 Period 1 (BWT gain for 3 day period in grams)
4DAYWK4B	- Week 4 Period 2 (BWT gain for 4 day period in grams)
7DAYWK5	- Week 5 (BWT gain for 7 day period in grams)
D1	- 1 hour/day
D2	- 4 hours/day
DL <sub>CO</sub>	- diffusion capacity for carbon monoxide (ml/min x tor)
EDTA	- ethylenediaminetetraacetic acid
EOS	- eosinophils (percent leukocytes counted)
EXP	- post-exposure

ATTACHMENT  
(CONTINUED)

LIST OF ABBREVIATIONS

F1	- two exposures/week
F2	- four exposures/week
FCWEEK1A	- average daily food consumption in Week 1 Period 1
FCWEEK1B	- average daily food consumption in Week 1 Period 2
FCWEEK2A	- average daily food consumption in Week 2 Period 1
FCWEEK2B	- average daily food consumption in Week 2 Period 2
FCWEEK3A	- average daily food consumption in Week 3 Period 1
FCWEEK3B	- average daily food consumption in Week 3 Period 2
FCWEEK4A	- average daily food consumption in Week 4 Period 1
FCWEEK4B	- average daily food consumption in Week 4 Period 2
FCWEEK5A	- average daily food consumption in Week 5 Period 1
FCWEEK5B	- average daily food consumption in Week 5 Period 2
FEF75	- forced expiratory flow at 75% of remaining FVC (ml/sec)
FEF50	- forced expiratory flow at 50% of remaining FVC (ml/sec)
FEF25	- forced expiratory flow at 25% of remaining FVC (ml/sec)
FEV50	- forced expiratory volume at 50 msec of expiration (ml @ 50 msec)
FEV100	- forced expiratory volume at 100 msec of expiration (ml @ 100 msec)
FEV200	- forced expiratory volume at 200 msec of expiration (ml @ 200 msec)
FEV400	- forced expiratory volume at 400 msec of expiration (ml @ 400 msec)
FOB	- frequency of breathing (breaths/min)
FVC	- forced vital capacity (ml)
GLU	- glucose (milligrams/deciliter serum)
H <sub>2</sub> O	- water
HCT	- hematocrit (percent)
HGB	- hemoglobin (grams/liter serum)
HighConc	- graphite @ 200 mg/m <sup>3</sup>
IM NEU	- immature neutrophils (percent leukocytes counted)
l/min	- liters per minute
LAV	- designated for pulmonary lavage
LowConc	- graphite @ 100 mg/m <sup>3</sup>
LUNGBWT	- lung to body weight ratio (x 100)
LYM	- lymphocytes (percent leukocytes counted)
LYMPH	- lymphocytes (percent leukocytes counted)



ATTACHMENT  
(CONTINUED)

LIST OF ABBREVIATIONS

MAT NEU	- mature neutrophils (percent leukocytes counted)
MCH	- mean corpuscular hemoglobin (picograms)
MCHC	- mean corpuscular hemoglobin concentration (percent)
MCV	- mean corpuscular volume (cubic microns)
mg/m <sup>3</sup>	- milligrams/cubic meter
ml/sec	- milliliters/second
mm	- millimeter
MMAD	- mass median aerodynamic diameter
MMEXF	- mean mid-expiratory flow (ml/sec)
MONO	- monocytes (percent leukocytes counted)
msec	- millisecond
N	- number
NEUT	- neutrophils (percent leukocytes counted)
NH <sub>4</sub> Cl	- ammonium chloride
	- sodium (milliequivalents/liter serum)
NRBC	- nucleated red blood cells (number/100 white blood cells)
ORNL	- Oak Ridge National Laboratories
PATH	- designated for pathology, clinical pathology and (recovery rats only) food consumption
PERVCELL	- percent viable cells (proportion viable)
Pes	- esophageal pressure (cm H <sub>2</sub> O)
PEXF	- peak expiratory flow (ml/sec)
PF	- designated for pulmonary function tests
PHAGO	- phagocytosis (counts per minute)
PHOS	- inorganic phosphate (milligrams phosphate/deciliter serum)
PLT	- platelet count (thousands of cells/cubic millimeter blood)
PosCont	- crystobalite @ 200 mg/m <sup>3</sup>
PROTEIN	- lavage fluid protein (micrograms/milliliter)
RBC	- red blood cell count (millions of cells/cubic millimeter blood)
REC	- post-recovery
RH	- relative humidity
Rl	- resistance (cm H <sub>2</sub> O/ml x sec)
RV	- residual volume (ml)

ATTACHMENT  
(CONTINUED)

LIST OF ABBREVIATIONS

SD	-	standard deviation
SDH	-	sorbitol dehydrogenase (international units/liter serum)
STDPHAGO	-	standardized phagocytosis (counts per minute)
TBA	-	total bile acids (micromoles/liter serum)
Te	-	expiratory time (sec)
Ti	-	inspiratory time (sec)
TLC	-	total lung capacity (ml)
TOTVCELL	-	total viable cells
TOTCELL	-	total cells
TP	-	total protein (grams protein/deciliter serum)
TRIG	-	triglycerides (milligrams/deciliter serum)
ul	-	microliter
VC	-	vital capacity (ml)
VCpv	-	vital capacity (pressure-volume derived) (ml)
VE	-	minute volume (ml/min)
Vemax	-	maximum flow during tidal expiration (ml/sec)
Vimax	-	maximum flow during tidal inspiration (ml/sec)
VT	-	tidal volume (ml)
VPEXF	-	volume at PEXF (ml)
WBC	-	white blood cell count (thousands of cells/cubic millimeter blood)